INTEGRATED STRIPE RUST MANAGEMENT OF BREAD WHEAT

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ABSTRACT

Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, affects wheat production in Canada. Two effective strategies to control this disease are the deployment of cultivars with stripe rust resistance, and the use of fungicides when susceptible and moderately resistant cultivars are grown under high stripe rust risk conditions.

The effect of fungicide application timing on hard red spring wheat cultivars varying in stripe rust resistance was determined at two seeding dates in central Saskatchewan from 2012 to 2016. Under high disease pressure at a mid-May seeding date, a single fungicide application at the mid-flower growth stage of wheat decreased disease severity to 26% compared with the unsprayed control at 87% for the susceptible cultivar 'AC Barrie'; the magnitude of the response was somewhat less for the moderately resistant cultivar 'CDC Imagine'. There was a significant yield increase of 59% when the fungicide was applied to the susceptible cultivar at mid-flower. Similar effects of fungicide application were observed for protein content, test weight and thousand-kernel weight. Furthermore, at a later seeding date (early June) fungicide application at stem elongation and at mid-flower growth stages of wheat had the same positive effect of reducing stripe rust symptoms from 87% to 51 and 54% and increasing yield by 53 and 46%, respectively for the susceptible cultivar "AC Barrie'. A fungicide application had no effect on the stripe rust resistant cultivar 'Lillian' at either seeding date; however, it did reduce leaf-spotting diseases on this cultivar. The study demonstrated that a single fungicide application reduces stripe rust severity, increases yield and improves grain quality in the susceptible and moderately resistant wheat cultivars used in this experiment at the mid-May seeding date. At the early June seeding

date, fungicide reduced disease severity of the susceptible and moderately resistant cultivars, but increased yield only for the susceptible cultivar.

Spelt (*Triticum aestivum* ssp. *spelta*), a sub-species of wheat, has been included in varietal development programs because its unique genetic composition makes it easy to cross with bread wheat to introgress desirable traits such as improved grain quality and stripe rust resistance. It has been found that spelt carries race-specific resistance gene *Yr*5, which confers resistance to all known *P. striiformis* f. sp. *tritici* races in North America. The stripe rust resistance of two spelt genotypes, CDC Silex and 10Spelt17, was studied by analyzing populations from crosses with the susceptible bread wheat cultivar ‘Avocet’. Based on growth chamber testing, the adult plant resistance of these spelt genotypes was shown to be conferred by at least two genes.

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DEDICATION

To Juan my amazing husband, and to my kids Fabiana and Maximilian, with you by my side everything is possible even if it takes longer than expected. And to my mom, dad and sisters who supported me to live with plants and science by my side. Love you all.

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LIST OF ABBREVIATIONS

AC	Agriculture and Agri-food Canada
ANOVA	Analysis of variance
APR	Adult-plant resistance
ASR	All-stage resistance
CDC	Crop Development Centre
CPRS	Canada Prairie Spring Red
CV	Cultivar
CWRS	Canada Western Red Spring
CWRW	Canada Western Red Winter
DMI	Demethylation-inhibitors
FAO	Food and Agriculture Organization
g	Grams
GS	Growth stage
Ha	Hectare
hL	Hectoliter
IT	Infection type
Kg	Kilograms
MR	Moderately resistant
PNW	Pacific Northwest
Pst	*Puccinia striiformis* f. sp. *tritici*
QoI	Quinone outside inhibitor
R	Resistant
SAS	Statistical Analysis Software

SK	Saskatchewan
S	Susceptible
TW	Test weight
TKW	Thousand kernel weight
µl	Microliter
USDA	United States Department of Agriculture
Yr	Yellow rust/stripe rust resistance

CHAPTER 1

1. INTRODUCTION

Stripe rust of wheat (*Triticum aestivum* L.) caused by the fungus *Puccinia striiformis* Westend. f. sp. *tritici* (*Pst*) is an important disease in many countries when favorable weather conditions occur (Chen 2005; Chen et al. 2014). The disease is also known as yellow rust because the symptoms appear as narrow, yellow-orange stripes of pustules called uredia, which are parallel to the leaf veins on adult wheat plants. The first report of stripe rust in Manitoba and eastern Saskatchewan was in 2000, and serious epidemics took place in 2006 and 2011 in western Canada causing yield losses of up to 35% in susceptible cultivars (Fetch et al. 2011; Brar and Kutcher 2016).

The most common control measures for stripe rust are the use of resistant wheat cultivars and the application of foliar fungicide. Cultivar resistance is considered all-stage resistance (ASR), which is usually race-specific and effective at both seedling and adult plant growth stages, or adult plant resistance (APR), which is generally non-race specific (Line and Chen 1995; Chen 2005; Luo et al. 2009). Foliar fungicide application to control stripe rust in susceptible or moderately resistant cultivars is an important management strategy for growers and several studies in other countries have been conducted to estimate yield loss and to reduce unnecessary use of fungicides (Chen 2007).

1.1 Hypotheses

Three hypotheses were developed for the two projects of this thesis:

1. Foliar fungicide application at mid-flowering (first anthers visible) is the optimum time to control stripe rust on susceptible bread wheat cultivars.
2. The benefit of foliar fungicide will vary among wheat cultivars depending on their resistance to stripe rust.
3. Adult plant stripe rust resistance in crosses of spelt *(Triticum aestivum* ssp. *spelta*) with bread wheat (*T. aestivum)* is due to ASR genes and is simply inherited.

1.2. Objectives

1. To assess the effectiveness of tebuconazole fungicide (Folicur® 250 EW) to control stripe rust when applied at three crop growth stages on three bread wheat cultivars representing a range of resistance to stripe rust in field plot experiments at two seeding dates, at multiple site-years.

2. To determine the inheritance of adult plant resistance to stripe rust in two crosses of spelt (stripe rust resistant) x bread wheat (cv ‘Avocet’, stripe rust susceptible) using one isolate of *Pst* under controlled conditions.

CHAPTER 2

2. LITERATURE REVIEW

2.1 Wheat

2.1.1 *Triticum* spp.: Importance and Biology

Domestication of cereals is an example of human impact on the evolutionary processes of speciation, natural selection, and adaptation (Gustafson et al. 2009).

Wheat describes a number of species of the genus Triticum, of three ploidy levels: diploids (einkorn) *T. monococcum* and *T. urartu,* all with the genome AA consisting of 7 pairs of chromosomes; tetraploids (emmer, durum, polish, rivet and persian wheat) *T. turgidum* and *T. timopheevii* with the genome AABB and 14 pairs of chromosomes; and hexaploids (spelt, bread, club and Indian shot wheat) *T. aestivum* with the genome AABBDD and 21 pairs of chromosomes (Knott 2012; McFadden and Sears 1946; Dvorak et al. 2011; Bonjean and Angus 2011).

The diploid *T. urartu* is the A genome donor to all wheat species; this diploid species was first found in Greece by Boissier in 1884 and later identified in southwestern Iran, northern Iraq, the Transcaucasia, eastern Lebanon, southeastern Turkey, western Syria and the Mediterranean (Bonjean and Angus 2011; Gustafson et al. 2009). The origin of the B genome is controversial, but some evidence suggests that

Aegilops speltoides Tausch (2n=2x=14) is the donor of this genome, not just for tetraploid wheat but also for hexaploid (Kerber and Rowland 1974).

The hexaploid wheats were created by the hybridization of *A. tauschii*, the male donor of the D genome, and *T. turgium* L. the female donor with the genome AABB (Matsuoka 2011; McFadden and Sears 1946). It is believed that they originated in Afghanistan, Turkmenistan and Transcaucasia approximately 10,000 years ago (Dvorak et al. 1998). Most species of the three ploidy groups have been cultivated, although only *T. turgidum* ssp. *durum* and *T. aestivum* ssp. *aestivum* are grown extensively and make up close to 90% of the cultivated wheat in the world (Mehta 2014).

2.1.2 *Triticum aestivum* ssp. *spelta* (Spelt)

Hexaploid *T. aestivum* ssp. *spelta* (spelt) is a hulled or covered wheat with adherent glumes and a brittle rachis, compared with common wheat *T. aestivum* whose kernels are free threshing (Dorval et al. 2015). Several theories on the origin of spelt have been suggested, one of which was that it originated from the spontaneous hybridization of *T. diccocoides* and *A. tauschii* in southwest Asia and then Europe. Another theory is that spelt originated in Europe from spontaneous crosses between *T. aestivum* and *T. diccocum* (Dvorak et al. 1998, Kerber and Rowland 1974). As *A. tauschii* is hulled, McFadden and Sears (1946) concluded that a hulled spelt is the ancestral form of the free-threshing hexaploid wheat. However, the discovery at archeological sites of a free-threshing hexaploid wheat before spelt has increased speculation about the ancestral position of spelt to free threshing wheat (Dvorak et al.

1998). Spelt is associated with common wheat because of it equivalency on protein content and higher nutritional levels (Wiwart et al. 2012).

Spelt was an important cereal crop cultivated in Europe in the 1930s when it made up almost 40% of the wheat growing area. Nowadays it is grown on a limited scale in Europe and Asia (Kema 1992b) and the United States (Stallknecht et al. 1996; Dvorak et al. 2006). There is renewed interest in spelt wheat as a low input crop for organic production. Also, its natural resistance against several fungal pathogens demonstrates its potential as a stripe rust resistance source (Dorval 2015; Kema 1992a). Several studies of spelt accessions since the 19th century have shown complete resistance to stripe rust even in years with high disease pressure; all these accessions carried the gene *Yr5* (Kema 1992a). This single dominant gene confers resistance to almost all known races of stripe rust in North America, but not in Australia, and it has been used to breed for resistance to the disease. The gene *Yr5*, in combination with other seedling and adult plant resistance genes, can provide durable resistance to wheat cultivars (Yan et al. 2003).

2.1.3 Wheat Production in the World and in Canada

Wheat along with rice and corn is one of the most important crops for human consumption in the world (Peng et al. 2011; Evans et al. 1981). Worldwide, approximately 220 million hectares of wheat are cultivated annually producing approximately 700 million tonnes (Balfourier et al 2019). It is an important source of carbohydrates and essential nutrients and delivers 15% of the calories consumed daily. In addition, it is easy to store and process into flour. Dough produced from bread wheat flour has unique viscoelastic properties and the starch is easily digested, as is the protein (Curtis et al. 2002). In Canada, wheat is used to make bread, a wide range of noodles, pasta, couscous, and it is used as feed for livestock.

The major wheat producers and exporters are Argentina, Australia, Canada, the European Union, Kazakhstan, the Russian Federation, Ukraine and the United States (FAO.org 2019). In 2018, over 10 million hectares of wheat were seeded in Canada, and the western Canadian provinces (Manitoba, Alberta, and Saskatchewan) accounted for over 9.5 million hectares with total production of 29 million tonnes (Statistics Canada 2019).

The Canada Western Red Spring (CWRS) bread wheat market class represents the most widely grown of all wheat market classes, accounting for 5.9 million hectares of western Canadian wheat and close to 19.7 million tonnes in 2018 (Statistics Canada 2019). The CWRS wheat cultivars command a premium price in the world market, which has led to high production (McCallum and DePauw 2008).

2.2 *Puccinia striiformis* f. sp. *tritici*, Causal Agent of Stripe Rust

2.2.1 History and Impact

Stripe rust of wheat caused by the fungus *P. striiformis* Westend. f. sp. *tritici* Eriks. (*Pst*), (McIntosh 1992) is an important disease of wheat. Globally, stripe rust has been reported from approximately 60 countries (Chen 2005). It is believed that the centre of origin of *Pst* is Transcaucasia, where grasses were the first hosts (Chen et al. 2014). The pathogen arrived in North America sometime before 1915 (Chen et al. 2002) and in Canada in 1926 (Su et al. 2003). It was reported in Germany and the

Netherlands in 1921 (Zadoks 1961), China in 1949 (Wan et al. 2007), Australia in 1979 (Wellings and McIntosh 1990), and in South Africa and the Middle East in 1996 (Boshoff et al. 2002).

In severe epidemics, stripe rust causes substantial yield losses, primarily by decreasing plant vigor, reducing kernel number and size and causing reduced germination. Yield loss caused by stripe rust can be as much as 100% depending on the susceptibility of the cultivar, particularly if the disease develops early in the season, which may result in several cycles of infection in the same growing season (Chen 2005). Several stripe rust epidemics in wheat growing countries have had major economic impact, for instance in New Zealand an epidemic in 1980-1981 caused yield losses of up to 60%, and in Australia between 1983-1986 yield losses were as much as 80% in some crop fields (Murray et al. 1994). In the United States, several epidemics have occurred causing up to 70% yield loss in 1960, 2000 and 2003 in several farmers' fields (Chen 2005; Chen et al. 2002; Wellings 2011).

In 2005 in southern Alberta, stripe rust of wheat became epidemic, reaching disease severities of 100% in the most affected crops and resulting in premature ripening and the development of uredia in-between kernels and glumes (McCallum et al. 2007). Stripe rust symptoms have been detected in certain susceptible Canadian wheat classes including Canada Western Red Winter (CWRW), Canada Western Red Spring (CWRS) and Canada Prairie Spring Red (CPRS) (Randhawa 2012; Puchalski and Gaudet 2010). Major epidemics in western Canada that took place in 2006 and 2011 caused yield losses up to 35% in susceptible cultivars (Kutcher et al. 2012; Fetch et al. 2011).

In North America, stripe rust is frequently detected in the Pacific Northwest (PNW) of the USA, where conditions are often optimal for the development of the disease. Wheat production in western Canada is at risk as it is believed that stripe rust spores blow into the area from the PNW and the Puccinia pathway, where the pathogen undergoes the sexual reproductive phase on its alternate host (Jin et al. 2010; Chen 2005; Brar and Kutcher 2016). Another risk factor seems to be the overwintering capacity of *Pst* as is the case in southern Alberta where *Pst* mycelium within winter wheat tissue can survive and create hot spots in a field early in the growing season. This situation can lead to early infection of wheat, resulting in the generation of a great amount of spore production (Lyon and Broders 2017). There are multiple reports of the overwintering potential of stripe rust when spring wheat is seeded near winter wheat generating a green bridge. For example, Kumar et al. (2013) reported that overwintering of *Pst* resulted in higher disease severity in spring wheat that was seeded near infected winter wheat than a crop seeded near another spring wheat crop.

In 2000, *Pst* was reported for the first time in Manitoba and eastern Saskatchewan (Kutcher et al. 2012; Fetch et al. 2011). In the same year *Pst* occurred in at least 20 states and was the most widespread and severe epidemic in the United States (Chen et al. 2002; Chen 2005). New and more aggressive races are now common in the PNW (Markell and Milus 2008). The new *Pst* races are adapted to warmer temperatures and are more aggressive than previously reported races (HovmØller et al. 2010). For instance, Milus et al. (2009) found that isolates collected since 2000 have shorter latent periods and spore germination occurs more rapidly at warmer temperatures than isolates collected before 2000.

2.2.2 Life Cycle

Wheat rusts are biotrophic organisms of the phylum *Basidiomycota*, class *Urediniomycetes*, order *Uredinales,* family *Puccinaceae*, and genus *Puccinia* (Chen et al. 2014). Stripe rust of wheat caused by *Pst* belongs to a special form or *forma specialis* (f. sp.) *tritici.* There are other *formae speciales* that infect specific hosts such as barley (*P. striiformis* f. sp. *hordei*), and rye (*P. striiformis* f. sp. *secalis*) (Wellings 2007).

Puccinia striiformis is heteroecious and macrocyclic (Fig. 1) and requires two different hosts to complete its life cycle, although it completes the asexual infection cycle several times within a season (Roelfs et al. 1992). *Puccinia striiformis* f. sp. *tritici* (*Pst*) can produce five types of spores: urediniospores, teliospores and basidiospores in wheat and pycniospores and aeciospores on alternate hosts.

Urediniospores are single celled and are released from the uredia; they can be carried by the wind short or long distances spreading the pathogen locally or over large geographical areas. Urediniospores land on the leaves and germination occurs within a few hours under favourable temperatures (0 to 15°C) (Roelfs et al. 1992). Once on the leaf a germ tube grows toward the stomata and forms an appressorium with a penetration peg that enters through the stomata and into the intercellular space of the leaf where the infective hyphae will spread, colonizing and forming the haustorium between 12 to 24 hours (Hu and Rijkenberg 1998). The haustorium is responsible for obtaining nutrients from the living host cells. The fungus will continue to spread within the leaf for approximately 14 days post inoculation, when the first sign of uredinial development becomes evident. The uredia erupt through the leaf surface to release urediniospores.

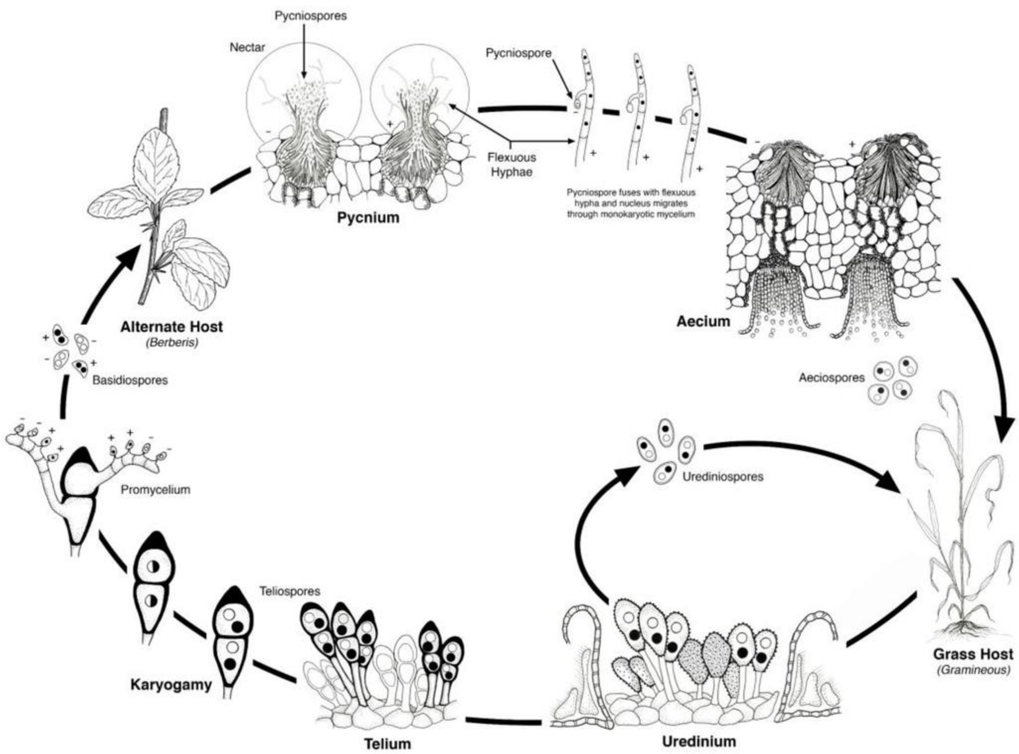

Fig. 2.1. Life cycle of *Puccinia striiformis* f. sp. *tritici*. Adapted from the *Puccinia graminis* f. sp. *tritici* life cycle (Kolmer 2013). Original illustration from Jacolyn A. Morrison at the USDA-ARS Cereal Disease Laboratory, St Paul, MN, USA. (Kolmer 2013).

The teliospores of *Pst* are two-celled structures produced in telia. During teliospore formation, karyogamy occurs between each haploid cell to form a diploid nucleus, which undergoes meiosis to form a promycelium from which four basidiospores are produced. The basidiospores lack the ability to re-infect wheat, but rather infect the alternate host to complete the sexual phase. The basidiospores are wind-blown to the alternate host and germinate forming another infective structure on the upper leaf surface. These are called pycnia, within which pycniospores are produced. Pycnia are of two mating types that must cross fertilize to give rise to a new structure called aecia on the lower leaf surface of the alternate host, in which aeciospores are produced, which have the capacity to re-infect wheat (Bolton et al. 2008).

Basidiospores of *Pst* can only infect certain species of *Berberis* spp. as: *B. chinensis, B. holstii, B. koreana* and *B. vulgaris* and Oregon grape (*Mahonia aquifolium)* (Jin et al. 2010; Wang and Chen 2013). The eradication of the alternate host (barberry) eliminates sexual reproduction of *Pst*.

2.2.3 Epidemiology

Infection of wheat plants by *Pst* can occur anytime from plant emergence until maturity as long as green tissue is available (Chen 2005). Stripe rust symptoms are usually observed after the tillering stage. The first symptoms appear on leaves as narrow, yellow-orange rows of pustules, which are the uredia that are parallel to the leaf veins, and on the leaf sheaths, glumes and awns of susceptible wheat cultivars. On seedlings, the uredia emerge on infected tissue in all directions, potentially covering the entire leaf (Chen et al. 2014).

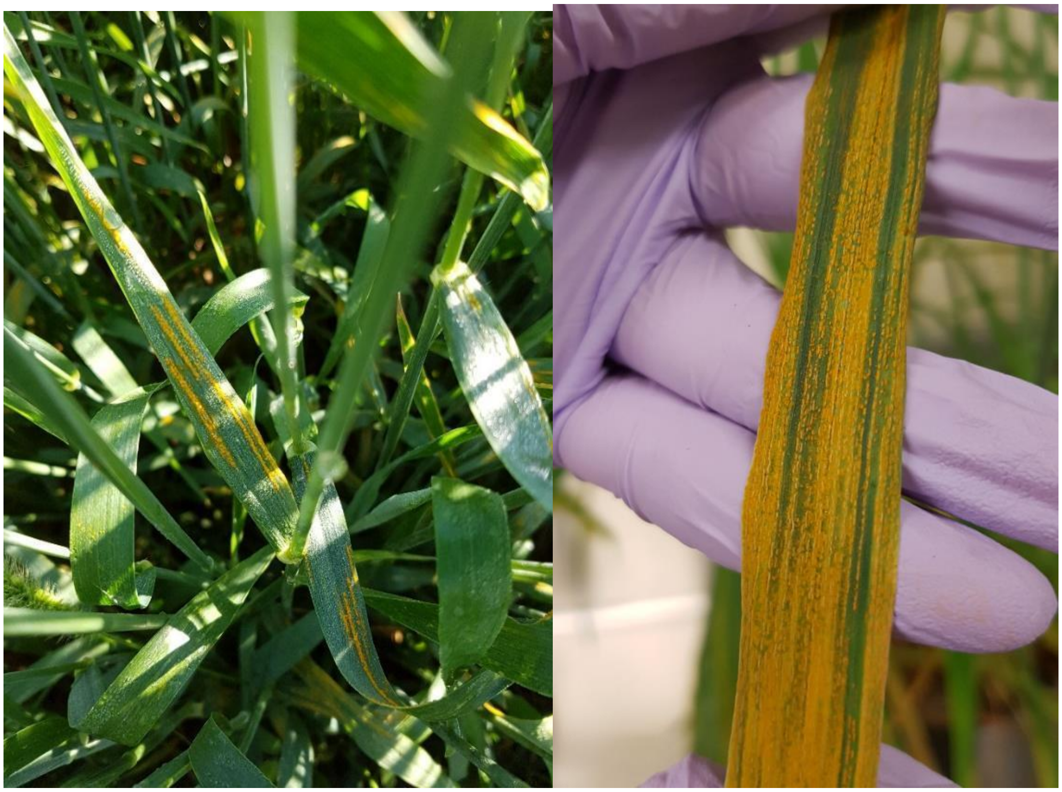

Fig. 2.2 Early symptoms of stripe rust on wheat leaves in the field. Adult plant infection on a susceptible cultivar. Photo credit J.T. Vera.

The spores of stripe rust require moisture to germinate and infect in the form of dew or precipitation for a minimum continuous leaf wetness period of between 4 to 6 hours. Urediniospores are unable to survive when the wet period is interrupted during germination and penetration. Conversely, un-germinated, dry spores are not affected by dry conditions (De Vallavieille-Pope et al. 1995). Rain can also disperse spores by direct impact or by splashing, resulting in spread to upper plant parts and to neighbouring plants (Chen 2005).

Another factor that can influence the development of stripe rust is temperature. It can affect spore germination, infection, latent period, spore survival and host resistance.

Cool temperatures are favorable for the development of *Pst*, leading to early disease development compared with the other rusts or leaf spot diseases, and can increase infectivity throughout the season (Rapilly 1979). The optimum temperature for *Pst* is 11°C and the highest rate of urediniospore germination occurs between 5 to 14°C, with a maximum limit of 21°C (Fetch et al. 2011). Sporulation is a function of relative humidity, which needs to be >50% but can be inhibited by liquid water (Hau and De Vallavieille-Pope 1998). Temperature can affect winter survival. Temperatures below -10°C may pause the development of the pathogen and cause winter kill of infected winter wheat tissue (Chen 2005; Rapilly 1979). Another factor that plays a major role in the spread of the pathogen is the wind, which dries the urediniospores and reduces germination and infection. *Pst* is well adapted for long-distance dispersal either by short or long-distance movement within the same growing season (Chen et al. 2014).

2.3 Control of Stripe Rust of Wheat

The occurrence of stripe rust is usually associated with the capability of the pathogen to overwinter, which can lead to early infection and damage to spring wheat cultivars. For these reasons, it is important to control stripe rust. The most common control measures are the use of resistant wheat cultivars and the application of foliar fungicides (Chen 2005).

2.3.1 Cultivar Resistance

2.3.1.1 Sources of stripe rust resistance

The most beneficial, relatively economical and environmentally friendly method to control stripe rust is the use of resistant cultivars (Kumar et al 2012; Line and Chen 1995). Wheat breeders are always looking for sources of germplasm that can be used to improve resistance to stripe rust.

There are several sources of stripe rust resistance: one source is the use of commercial cultivars, which carry effective genes for the control of stripe rust and are currently available. Another source it is the use of old cultivars that carry effective genes specific for certain geographic locations. Other sources are species that can be intercrossed with common wheat cultivars, for example, spelt wheat, which has been crossed with common bread wheat to transfer *Yr*5 for resistance to stripe rust. Wild wheat relatives can be used as sources of resistance; however, their use can transfer undesirable botanic and agronomic traits and slow the development of new resistant cultivars in a breeding program. However, some genes effective against stripe rust have been successfully extracted from wild relatives and are used in commercial cultivars such as *Yr5, Yr9, Yr10, Yr15, Yr17, Yr24/26, and Yr76* (Wang and Chen 2017).

Currently there are close to 80 genes officially designated as *Yr* genes in wheat; most of these genes are race-specific and follow the gene-for-gene theory of host-pathogen interactions (Cloutier et al 2007; McIntosh 1992).

2.3.1.2 All stage resistance

Specific resistance is referred to as ASR, which usually means that resistance to the pathogen is effective at the seedling stage and throughout the life of the plant. Breeding for specific resistance against stripe rust is inherited according to Mendelian genetics (Mehta 2014; Line and Chen 1995). This type of resistance was first observed by Biffen (1905), who reported that resistance in wheat segregated in a 3 resistant: 1 susceptible ratio, indicating it was governed by a single dominant gene or simple combinations of single genes. This type of resistance can be easily observed as a low infection type (IT) or no rust symptoms in seedling tests. However, the durability of this resistance is usually limited due to pathogen evolution over time, sometimes as short as 3-4 years after the release of a resistant cultivar (Line and Chen 1995). There are several examples of race-specific disease resistance breakdown. Virulent stripe rust races overcame the resistance of the gene *Yr10* for the first time in 1968, and in 2005 the breakdown of this gene in the PNW of the USA occurred rapidly (Line and Qayoum 1992; Chen et al. 2010). In western Canada in 2010, during the stripe rust epidemic in southern Alberta, races overcame *Yr10*, which resulted in heavy infection of cultivars carrying this gene such as ‘Radiant’ (Puchalski and Gaudet 2011; Kutcher et al. 2012). In Saskatchewan, the first report of a race virulent on *Yr10* was in 2013 (Brar and Kutcher 2016); however, the ASR genes *Yr5* and *Yr15* are still very effective in Canada and in most countries where the disease is present (Sharma-Poudyal et al. 2013). There are tools or strategies that may prolong the effectiveness of ASR to the disease when new races appear.

Using multiline cultivars or gene pyramiding, it is possible to extend the life-span of ASR. One example of a multiline cultivar is cv. 'Rely', which is composed of more than ten different resistance genes (Chen 2007). This cultivar, which is cultivated in the PNW of the USA, has maintained resistance since 1991. Another strategy to increase the lifespan of stripe rust resistance genes is to use gene pyramiding. This method consists of the addition of several resistance genes into one variety using molecular markers to facilitate gene pyramiding. This has been used to add genes that are individually effective against *Pst* and can prolong resistance to stripe rust.

2.3.1.3 Adult plant resistance

Another type of resistance is associated with quantitative resistance regulated by major genes and/or minor genes, which confer partial resistance or a slow-rusting phenotype. Some sources of quantitative resistance are characterized by their response to temperature, as high-temperature adult plant (HTAP) resistance genes. As the plant grows and the temperature increases, the effectiveness of the genes increase, in contrast to the reaction of the same genes at the seedling stage and at low temperatures (Wang and Chen 2017).

One of the characteristics of APR is that most are non-race specific and have been effective for more than 30 years; although, some of the APR genes can be race specific such as *Yr11*, *Yr12*, *Yr13* and *Yr14* (HovmØller 2007; Line and Chen 1995). Among the classes of western Canadian wheat, Canada Western Red Spring (CWRS) cultivars have a range of reactions from susceptible to highly resistant to stripe rust. The presence of genes such as *Yr11 Yr14*, *Yr16*, *Yr18*, *Yr29*, *Yr30* and *Yr36* is attributed to

APR (Randhawa et al. 2012; Chen 2014). Those cultivars carrying the APR gene *Yr18* have a moderate level of resistance, not just to stripe rust, but also to other biotrophic pathogens including leaf rust (for which *Yr18* is also known as *Lr34*) and powdery mildew (*Yr18* = *Pm*38). The *Yr18* gene also conditions leaf tip necrosis (*Ltn1*), which is a visual marker to identify lines that may carry APR genes. Other examples of this composite resistance are *Lr46/Yr29/Pm39/Ltn2*, and *Lr67/Yr46* (Singh et al. 1992). Another APR gene effective for resistance to stripe rust is *Yr36*, which was isolated and characterized from wild *T. dicoccoides* and is linked to the high grain protein content gene *Gpc-B1*. (Randhawa et al. 2012; Yuan et al. 2012).

The pyramiding of APR genes into cultivars is believed to result in highly durable rust resistance, but little is known of this genes benefits, or the interaction of these types of genes with ASR (Hiebert et al. 2010). Pyramiding genes can provide higher levels of resistance than any one gene alone, as these genes are believed to act in an additive manner (Rosewarne et al. 2006).

2.3.2 Chemical control

The use of fungicides is a practical and sometimes necessary response to control stripe rust and mitigate yield loss on susceptible wheat cultivars and in some circumstances on moderately resistant cultivars (Chen 2007). There are several fungicides with a broad spectrum of disease control and efficacy at different plant stages that can protect the plant against the pathogen depending on the degree of disease severity. However, the use of pesticides to control diseases is not environmentally friendly, and can be expensive for the farmer. Furthermore, the pathogen can develop resistance to the active ingredient.

Using a resistant cultivar is the most inexpensive, environmentally friendly and efficient disease management strategy to minimize yield losses. The use of resistant cultivars can reduce yield losses by up to 90%. However, depending on yield potential, the price of wheat and the fungicide, even a relatively small percentage (10%) yield loss may be significant for a commercial grower; therefore, a fungicide application may be necessary (Chen 2014).

The first effective commercial application of fungicides to control *Pst* was in 1981 with the active ingredient triadimefon, which prevented millions of dollars of losses in the PNW of the USA (Line 2002). Nowadays, there are several fungicides registered to control stripe rust that consist of a number of active ingredients. Many are triazoles (flutriafol, propiconazole, tebuconazole, and triadimefon) (Table 2.1), all of which are in fungicide Group 3, the demethylation-inhibitors (DMI). These Group 3 DMI fungicides prevent the formation of sterols needed in fungal cell membranes. They are usually systemic to some degree as they penetrate the plant cuticle and are transported in the xylem (Murray et al. 2005; Chen 2014). The DMI fungicides inhibit the development of stripe rust in leaf tissues by altering the structure of the hyphae and haustoria and increasing vacuolation, causing changes in cell wall thickness, degeneration of the cytoplasm and breakdown of hyphae and haustoria (Han et al. 2006).

Triazoles are often combined with strobilurin fungicides, also known as Quinone outside inhibitor (QoI) fungicides. Strobilurins are assigned to Group 11 (azoxystrobin, kresoxim-methyl, pyraclostrobin, and trifloxystrobin). This type of fungicide was developed from isolates of *Strobilurus tenacellus,* a wood rotting mushroom fungus. Some QoI fungicides exhibit translaminar movement meaning that once applied the active ingredient is held on or within the cuticle of the leaf surface and xylem systemic (Vincelli 2002).

There are several foliar fungicides registered to control stripe rust of wheat in Canada (Table 2.1). And there are some co-formulations of triazoles and strobilurins are registered for use in Canada that are effective to control stripe rust and other foliar diseases of cereals, some of them are, azoxystrobin + propiconazole (Quilt®, Topnotch®, TrivaproA®), propiconazole + picoxystrobin (Cerefit®) (Chen and Kang 2017; Murray et al. 2005; Anonymous 2018).

Fungicide application timing is important for effective control of stripe rust; this is determined by the environmental conditions, the winter survival of the pathogen in each region and season, and the early development of the disease (Chen 2014). Disease severity is also affected by the susceptibility of the cultivar and previous fungicide used. Most of the fungicides registered to control stripe rust cannot be applied after flowering and some cannot be used 30-40 days before harvest (Chen and Kang 2017).

Some of these fungicides were tested using various application timings by several researchers in North America. In the 2000s, the use of foliar fungicides in Texas reduced yield losses by 41% compared with the unsprayed control (Reid and Swart 2004). In the PNW area in 2013, foliar fungicide applications at 10.5 on the Feekes scale (Large 1954) or mid-flowering stage (GS61) based on the BBCH wheat scale (Lancashire et al 1991) significantly reduced stripe rust severity on naturally infected wheat. Using azoxystrobin + propiconazole as active ingredients, disease severity was reduced by almost 93% on susceptible cultivars and yield increased by 15%. In that study, disease development was slow, and symptoms appeared late due to hot, dry environmental conditions (Chen 2014).

During the years 2016 and 2017, severe stripe rust epidemics occurred in the PNW. In 2016, stripe rust appeared in late May, which was earlier in the season than usual and the spring wheat was at the early tillering stage (GS20). Two fungicide applications were required to achieve a disease reduction of 92% and a yield increase of 40% on a susceptible cultivar compared with the unsprayed control (Chen and Kang 2017). In 2017, fungicide was applied when there was no sign of stripe rust at stem elongation or jointing (GS30). During that year, disease pressure was high in the PNW early in the season and the fungicide decreased disease severity by 83% on a susceptible cultivar and increased yield from 19 to 93% over the unsprayed control. Several other foliar fungicides with diverse active ingredients were evaluated that significantly reduced disease severity between 56 and 92% on the susceptible cultivar 'Avocet S' when the fungicides were applied before or at the flowering stage (GS 60) and resulted in increased yield (Chen and Kang 2017).

Other strategies suggested to reduce stripe rust are the use of seed treatment to delay the onset of the disease at seedling stages (Line 2002). Effective volunteer control is recommended because volunteer wheat provides a green bridge between seasons that facilitates overwintering of the pathogen and provides inoculum for the following growing season. The latter can be achieved using cultivars not prone to shattering and use of tillage or chemfallow. Excess irrigation should be avoided because moisture is essential for the development of the pathogen. With stripe rust conducive temperatures, spring wheat crops will be more vulnerable to infection. Therefore, it is important to grow resistant cultivars and use subsurface or drip irrigation instead of sprinkler irrigation (Chen and Kang 2017).

As stripe rust is adapting to changing environmental conditions and due to the lack of highly effective resistances in spring wheat cultivars, there is a need to investigate different ways to control this pathogen and the lack of highly effective and durable stripe rust resistance is a long-term issue for wheat breeding programs. The residual effects of defeated resistance genes in wheat could provide another tool to develop durable resistance with existing *Yr* genes and this study could provide much needed insight into the presence and effectiveness of this effect in the wheat-stripe rust pathosystem. And the use of fungicides with different active ingredients can control stripe rust on susceptible cultivars and reduce yield losses and it is a widely effective practice worldwide.

Table 2.1. Fungicides registered to control stripe rust in Saskatchewan 2019 (Anonymous 2018).

Trade name	**Active ingredient**	**Chemical family**	**Group**	**Rate (ml/ha)**	**Application timing according to label**
Acapela®	picoxystrobin 250 g/L	strobilurin	11	432 to 865	Prior to disease development to Flag leaf
Tilt 250*®	propiconazole 250 g/L	triazole	3	494	Tillering, stem elongation up to half emergence of the head
Caramba®	metconazole 90 g/L	triazole	3	494 to 692	Apply before the onset of the disease
Folicur®	tebuconazole 250 g/L	triazole	3	370 to 494	Apply when disease symptoms appear
Nexicor®	fluxapyroxad 30g/L; pyraclostrobin 125 g/L; propiconazole 125 g/L	carboxamide; strobilurin; triazol	3,7	494	Apply prior to disease development or at the onset of disease
Priaxor®	fluxapyroxad 1 67 g/L; pyraclostrobin 333 g/L	carboxamide; strobilurin	7,11	222 to 297	Apply prior to disease development or at the onset of disease symptoms
Prosaro®	prothioconazole 125 g/L; tebuconazole 125 g/L	triazole	3	803	Apply within the at least 75% of the heads are fully emerged to when 50% of heads are in flower
Quilt®	azoxystrobin 75g/L; propiconazole 125 g/L	strobilurin; triazole	3,11	751 to 1000	Apply between stem elongation and half head emergence
Topnotch®	azoxystrobin 143 g/L; propiconazole 124 g/L	strobilurin; triazole	3,11	529	Apply between stem elongation and half head emergence
Trivapro A**®	azoxystrobin 75g/L; propiconazole 125 g/L	strobilurin; triazole	3,11	988	Apply between stem elongation and head half emergence up until flag leaf stage
Twinline®	pyraclostrobin 130 g/L; metconazole 80 g/L	strobilurin; triazole	3,11	370 to 494	Apply prior to disease development or at onset of disease. Optimal timing is at leaf stage

*Other trade names: *Tilt 250E/Bumper 432 EC/Pivot 418 EC/Propel/Nufarm Propiconazole Fungicide/Propi Super 25 EC/Fitness*
**Trivapro A and B is a co-pack product

CHAPTER 3

3. FUNGICIDE APPLICATION AND CULTIVAR RESISTANCE ARE EFFECTIVE STRIPE RUST CONTROL STRATEGIES

3.1 Introduction

An effective method of stripe rust control is the use of resistant cultivars (Kumar et al 2012; Line and Chen 1995). It is an inexpensive, environmentally friendly and efficient disease management strategy to minimize yield losses. The use of stripe rust resistant cultivars can lead to a reduction of up to 90% in stripe rust severity and reduce yield losses by at least 20%. However, even a 20% yield loss caused by this disease may be significant for a commercial grower; therefore, a fungicide application may be beneficial even on resistant cultivars (Chen, 2014).

The use of fungicides is a practical and necessary response to stripe rust to mitigate yield losses on susceptible wheat cultivars and in some circumstances on moderately resistant cultivars (Chen, 2007). There are several fungicides with a broad spectrum of disease control that can protect the plant against the pathogen at different plant stages depending on the degree of disease severity. There are several fungicides registered to control stripe rust; the triazoles are group 3 demethylation-inhibitors (DMI) fungicides (flutriafol, propiconazole, tebuconazole, and triadimefon) commonly used for stripe rust control. The DMI fungicides prevent the formation of sterols that are needed in fungal cell membranes. These fungicides penetrate the plant cuticle, and are transported in the xylem making them systemic fungicides (Murray et al., 2005, Chen, 2014). The DMI fungicides inhibit the development of stripe rust in leaf tissues by altering the structure of the hyphae and haustoria and increasing vacuolation, causing changes in cell wall thickness, degeneration of the cytoplasm and breakdown of hyphae and haustoria (Han et al., 2006).

The timing of fungicide application is important for effective stripe rust control and the most appropriate timing depends on environmental conditions. Application may be warranted at an early stage of crop development if the pathogen has overwintered, and disease development begins early in the season (Chen, 2014). Relatively cool, damp conditions are conducive to stripe rust, which is common in the spring in North America. Disease severity is also affected by the susceptibility of the cultivar and the previous fungicide used. Most of the fungicides registered for control of stripe rust cannot be applied after flowering due to a requirement for a pre-harvest interval of 30-40 days (Chen and Kang, 2017).

3.2 Hypotheses and objective

3.2.1 Hypotheses

1) Foliar fungicide application at mid-flower (50% anthers visible) is the optimum time to control stripe rust on susceptible bread wheat cultivars.
2) The benefit of foliar fungicide will vary among wheat cultivars depending on their resistance to stripe rust.

3.2.2 Objective

To assess the effectiveness of tebuconazole fungicide (Folicur® 250 EW) to control stripe rust when applied at three crop growth stages on three bread wheat cultivars representing a range of resistance to stripe rust in field plot experiments at two seeding dates, over multiple site-years.

3.3 Materials and methods

3.3.1 Experimental locations and field trials

The field studies were conducted under a no-till seeding system from 2012 to 2016 at three sites in Saskatchewan: Pike Lake, SK at the Bayer CropScience Research Farm (51°49’24.672”N 106°46’17.184”W) from 2013 to 2016; Saskatoon, SK at the University of Saskatchewan East Sutherland Crop Research Farm (52°10’0.984”N 106°31’3.3744”W) from 2012 to 2016; and Melfort, SK at the Agriculture and Agri-food Canada Research Farm (52°49’05.2”N 104°35’31.3”W) in 2013 and 2014. The trials were usually established in fields with canola or pea stubble. Soil samples were collected at each site-year and fertilizer was applied based on recommended target yields for each site. At all sites prior to and after seeding, herbicides were applied to suppress weeds as necessary. Herbicides were applied pre-seeding, pre-emergence and before the 3 to 4 leaf stage of the wheat to control weeds.

The plot size was 2 x 8 m^2 at all locations and the seeding rate was 250 seeds per m^2. The plant density was measured two weeks after seeding by counting the number of seedlings in 1 m of two rows per plot. A spreader row consisted of a mix of stripe rust susceptible wheat cultivars ‘AC Barrie’ and ‘AC Morroco’ was grown between replicates and surrounding each trial.

To increase the stripe rust severity in the field, the spreader rows and borders were inoculated using a controlled droplet applicator (Herbeflex®), two or three times at the three leaf stage with a span of 4 days between inoculations with a Saskatchewan stripe rust mix of isolates (Table 3.1) suspended in light mineral oil (Bayol 55, Imperial Oil, Toronto, ON, Canada) at the 2-4 leaf stage (GS 12-14). After inoculation, the spreader rows were watered and covered with tarps for 24 hours to increase humidity around the plants under the tarp and promote infection. The trials at Saskatoon were irrigated periodically in the late afternoon during the growing season to maintain the high night-time humidity.

Table 3.1. Saskatchewan mix of isolates used for artificial inoculation at all site years. Characterization to race by Brar et al. (2016).

Isolate	Host	Year collected	Location	Race
W002	Wheat	2011	Denholm, SK	C-PST-16
W003	Wheat	2011	Kinley, SK	C-PST-2
W004	Wheat	2011	Hanley, SK	C-PST-1

3.3.2 Experimental design

Each site-year consisted of two trials: 1) a mid-May seeding date, and 2) an early June seeding date. Each trial was a split-plot design with 4 replications, with fungicide application treatments as the main plots and cultivars the sub-plot treatment factor.

The fungicide application timing treatments were at stem elongation, GS31 of the BBCH scale (Lancashire et al. 1991); mid-flower, GS 65; early milk, GS73; and a multiple application treatment that included applications at all three stages (GS 31, 65 and 73) to attempt a disease-free control; and an unsprayed control (Table 3.2). The commercial fungicide used was Folicur® 250EW, active ingredient tebuconazole, applied at a rate of 250 g per L of active ingredient per hectare. Three Canada Western Red Spring (CWRS) wheat cultivars were selected based on their stripe rust resistance: 'AC Barrie' - susceptible (S), 'CDC Imagine' - moderate resistant (MR) and 'Lillian' - resistant (R).

Table 3.2. Fungicide application treatment timings

Treatment	Description
1	Unsprayed control
2	Stem elongation (GS 31)
3	Mid-flower (GS 65)
4	Early milk (GS 73)
5	Multiple applications (GS 31+65+73)

3.3.3 Disease assessments

Disease severity was assessed on each plot before the first fungicide application plus a final assessment at the soft dough stage (GS 85). Leaf spot diseases were assessed on the Horsfall – Barratt scale (Horsfall and Barratt 1945) (Fig. 3.1). For stripe rust severity the modified Cobb scale, which describes the percentage of leaf tissue covered by stripe rust per leaf (Peterson et al.1948), was used to estimate the mean percentage of leaf tissue affected on 10 flag leaves and 10 penultimate leaves (Fig. 3.2).

Grade	% Diseased	% Healthy	Grade Formula (%)
0	0	100	1.17
1	0-3	97-100	2.34
2	3-6	94-97	4.68
3	6-12	88-94	9.37
4	12-25	75-88	18.75
5	25-50	50-75	37.50
6	50-75	25-50	62.50
7	75-88	12-25	81.25
8	88-94	6-12	90.63
9	94-97	3-6	95.31
10	97-100	0-3	97.66
11	100	0	98.62

Fig. 3.1. Leaf spots rating scale describing 12 grades based on diseased and healthy leaf area (Horsfall and Barratt 1945).

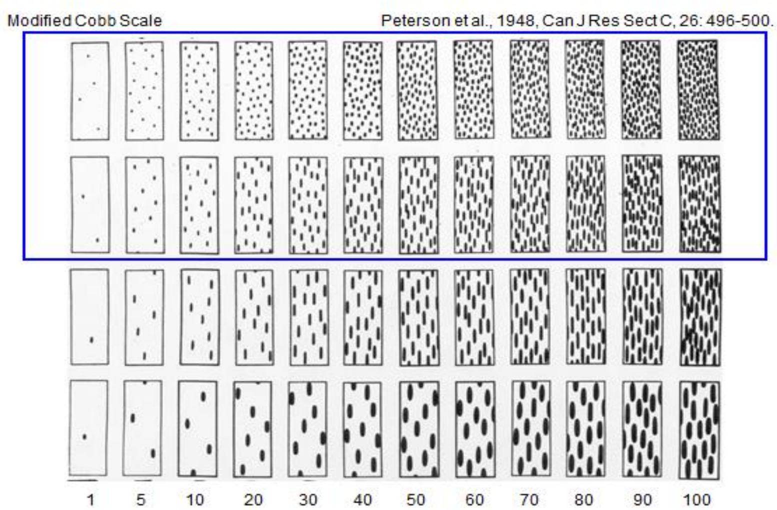

Fig. 3.2. Modified Cobb scale for stripe rust severity described as the percentage of leaf tissue covered by stripe rust pustules per leaf (Peterson et al.1948)

At maturity, each plot was harvested mechanically with a small plot combined and the grain bagged. Samples were air-dried for 24 hours or until the seed reached 14% moisture and then cleaned. After cleaning, the grain from each plot was processed to determine yield (kg ha^{-1}), test weight (kg/hL) and thousand kernel weight (g). A sub-sample of 250 g per plot was analyzed to determine the percent moisture content and 10 g were ground to determine protein content using a LECO® Nitrogen Food protein analyzer. Environmental conditions (precipitation and temperature) were recorded using meteorological stations inside the field trials and/or from Environment Canada data.

3.3.4 Data Analysis

Site-years were grouped according to severity of stripe rust. Levene's test was performed to determine the homogeneity of variances for the high and low stripe rust disease severity site-year groups. For the two groups using the MIXED procedure of SAS software 9.4 (SAS Institute Inc., Cary, NC, USA) with the fungicide application treatments and cultivars considered as fixed effects. Replications and replication by fungicide treatments effect were considered as random effects from each site -year, and for grouped data site-year, replication nested in site-years and replication by fungicide treatments nested in site-years were considered random effects for all variables. In cases where the ANOVA F-tests were significant for fixed effects, treatment means were compared using Tukey's test at a significance threshold of $P \leq 0.05$. Pearson's correlation coefficients were calculated using the CORR procedure of SAS for all variables in the low and high stripe rust severity groups.

3.4 Results

3.4.1 Environmental conditions

Precipitation was recorded for each growing season from May to September (Table 3.3). Saskatoon and Pike Lake during the years 2014 and 2016, and Melfort 2014 had above average rainfall at 230 and 390 mm compared with Melfort 2013, and Saskatoon and Pike Lake 2015, where precipitation was lower and ranged from 170 to 250 mm.

Table 3.3. Growing season precipitation (mm) between May and September of 2012 to 2016 at Saskatoon, Pike Lake and Melfort, Saskatchewan.

Site	Year	Precipitation (mm)					
		May	June	July	August	September	Total
Saskatoon	2012	148	107	93	63	1	412
	2013	16	118	36	15	1	184
	2014	61	95	45	19	11	230
	2015	6	20	85	58	51	221
	2016	43	47	77	70	25	261
Pike Lake	2013	60	103	78	15	40	296
	2014	50	106	115	98	22	391
	2015	-	30	50	41	50	173
	2016	-	-	-	-	-	-
Melfort	2013	19	98	103	12	18	250
	2014	24	167	39	58	9	298

The mean temperatures during the growing season were above normal during the years 2015 and 2016 at Saskatoon and Pike Lake, and near normal during 2012, 2013 and 2014 at all sites (Table 3.4).

Table 3.4. Growing season mean temperatures (°C) between May and September of 2012 to 2016 at Saskatoon, Pike Lake and Melfort, Saskatchewan.

Site	Year	Temperature (°C)					
		May	June	July	August	September	Mean
Saskatoon	2012	9.9	15.8	19.7	17.7	13.2	15.3
	2013	12.9	15.7	17.5	18.6	15.1	15.9
	2014	10.1	14.1	18.3	17.9	12.4	14.6
	2015	11.3	18.1	20.1	18.6	12.9	16.2
	2016	14.7	18.5	19.3	16.9	11.8	16.2
Pike Lake	2013	14	16.1	17.4	18.1	14.7	16.1
	2014	14.4	14.5	17.9	17.5	11.7	15.2
	2015	12.3	17.5	18.9	17.4	11.6	15.5
	2016	-	-	-	-	-	-
Melfort	2013	12.0	15.4	16.4	17.7	14.4	15.2
	2014	10	14.0	17.5	17.6	11.9	14.2

3.4.2 Mid-May seeding date

Based on the stripe rust severity of the unsprayed susceptible cultivar ‘AC Barrie’ the site-years were classified into ‘high’ or ‘low’ stripe rust severity groups (Table 3.5). The stripe rust severity control treatment of individual site-years comprising the high stripe rust severity group (referred to henceforth as the high group) ranged from 60 to 100%. Site-years where the unsprayed cultivars ‘AC Barrie’ had <13% stripe rust severity (e.g. no symptoms were observed at Melfort) were classified into the low stripe rust severity group (henceforth referred to as the low group). Disease severity of the unsprayed ‘AC Barrie’ treatment was 87% in the high group and 5% in the low group.

Table 3.5. High and low stripe rust severity site-year groups based on severity of the unsprayed treatment of ‘AC Barrie’ (susceptible) for the normal seeding date (mid--May) experiments.

Group	**Site-Year**
High stripe rust severity	Saskatoon 2012, 2013, 2014 and 2016
	Pike Lake 2014 and 2016
Low stripe rust severity	Saskatoon 2015
	Pike Lake 2013 and 2015
	Melfort 2013 and 2014

3.4.2.1 High stripe rust severity group

<u>Stripe rust severity (%)</u>

There was an interaction between cultivar and application timing (P<0.0001) in the high group (Table 3.6). Stripe rust severity for the susceptible cultivar ‘AC Barrie’ was 87% and was reduced to 58% when the fungicide was applied at stem elongation and to 26% when the fungicide was applied at mid-flower (Table 3.7). Fungicide applied at the early milk stage of ‘AC Barrie” resulted in a stripe rust severity of 77%, which was not different from the unsprayed control. As expected, the multiple fungicide application treatment had the lowest disease severity (11%). The moderately resistant cultivar ‘CDC Imagine’ had a stripe rust severity of 54% for the unsprayed fungicide treatment and a reduction to 28% was observed when fungicide was applied at stem elongation. Fungicide application at mid-flower of ‘CDC Imagine’ reduced disease severity to 14% and for the multiple application treatment to 4%. Stripe rust severity was very low for the resistant cultivar ‘Lillian’ for all fungicide treatments. The unsprayed treatment for ‘Lillian’ had a stripe rust severity of 1% and fungicide treated plots did not differ statistically from the unsprayed control. No differences among treatments were detected for this cultivar.

Table 3.6. *P* values from the analysis of variance for fixed effects of bread wheat cultivar (C), fungicide application timing (T) and their interaction (C x T) on stripe rust severity, leaf spot severity, yield, test weight (TW), thousand kernel weight (TKW) and protein content at all site-years.

	Group		Stripe rust	Leaf spot	Yield	TW	TKW	Protein content
Mid-May seeding date	High Stripe rust severity group	Cultivar (C)		<0.0001				
		Time (T)		<0.0001				
		C X T	<0.0001	NS	<0.0001	<0.0001	<0.0001	<0.0001
	Low stripe rust severity group	Cultivar (C)	NS	NS	0.0168	<0.0001	0.0348	<0.0001
		Time (T)	0.0445	0.0106	0.0003	0.0004	<0.0001	NS
		C X T	NS	NS	NS	NS	NS	NS
Early June seeding date	High Stripe rust severity group	Cultivar (C)		NS		<0.0001		
		Time (T)		<0.0001		0.0161		
		C X T	<0.0001	NS	<0.0001	NS	<0.0001	0.0019
	Low stripe rust severity group	Cultivar (C)		NS	0.0033	<0.0001	<0.0001	<0.0001
		Time (T)		0.0204	0.0028	<0.0001	<0.0001	0.001
		C X T	<0.0001	NS	NS	NS	NS	NS

"NS" = non-significant (P > 0.05).

Leaf spot disease severity (%)

There was no interaction between cultivar and fungicide application timing (P=0.0823) for leaf spot severity; however, differences among wheat cultivars and among application timings were detected (P<0.0001) (Table 3.6 and Table 3.8). The unsprayed treatment of the susceptible cultivar 'AC Barrie' had the highest leaf spot severity at 25%; and decreased when the fungicide was applied at mid-flower to 11%. The multiple fungicide application treatment resulted in a leaf spot disease severity of only 9% in ‘AC Barrie’. Furthermore, the cultivar ‘Lillian’ suffered somewhat greater leaf spot severity (22%) than ‘CDC Imagine’ (15%), but neither ‘Lillian’, nor ‘CDC Imagine’ were different from ‘AC Barrie’ (18%).

Yield (kg ha^{-1})

There was an interaction between application timing and cultivar (P<0.0001) for yield of wheat (Table 3.6). The yield of ‘AC Barrie’ was 4373 kg/ha when fungicide was applied at mid-flower, a 60% increase over the control treatment, which had a yield of 2733 kg ha^{-1} (Table 3.7). When the fungicide was applied at stem elongation a yield increase of 32% was observed (3595 kg ha^{-1}). As expected, the multiple fungicide application treatment had the highest yield of 4911 kg ha^{-1} in ‘AC Barrie’, although it was not statistically different from fungicide applied at mid-flower. No differences among fungicide application timing treatments at stem elongation, mid-flower, or early milk were detected for ‘CDC Imagine’, although yield of the treatment when fungicide was applied at mid-flower (3914 kg ha^{-1}) was much greater than the unsprayed treatment (2966 kg ha^{-1}). The mid-flower treatment was similar to the multiple application treatment (4248 kg ha^{-1}). No field differences among fungicide treatments were detected for ‘Lillian’.

Thousand kernel weight (g)

There was an interaction between application timing and cultivar (P<0.0001) for thousand kernel weight (TKW) (Table 3.6). The TKW of the stripe rust susceptible cultivar ‘AC Barrie’ had a mean of 32.6 g for the unsprayed control, which increased to 38.2 g when fungicide was applied at mid-flower and to 35.6 g when the fungicide was applied at stem elongation (Table 3.7). Fungicide applied at early milk stage resulted in a TKW of 34.9 g, which was similar to the unsprayed treatment and the fungicide applied at stem elongation. As expected, the multiple application treatment had the highest TKW (39.3 g). For the stripe rust moderately resistant cultivar, ‘CDC Imagine’, the multiple fungicide application treatment (38.7 g) and fungicide applied at mid-flower (38 g) differed from the unsprayed control (35.7 g) and the other two fungicide application treatments. The TKW for the unsprayed control of cultivar ‘Lillian’ (37.4 g) differed from fungicide applied at mid-flower (38.7 g) and the multiple application treatment (39.3 g).

Test weight (kg hL^{-1})

For this group of site-years, there was an interaction between application timing and cultivar ($P<0.0001$) (Table 3.6). The TW for the control treatment ‘AC Barrie’ was 77.5 kg hL^{-1} and it was increased to 79.9 kg hL^{-1}when the fungicide was applied at mid-flower to 80.1 kg hL^{-1} with the multiple application treatment (Table 3.7). The fungicide applications at stem elongation and early milk increased TW to 78.8 kg hL^{-1} and they differ from fungicide applied at mid-flower and the multiple application treatment. For the moderately resistant cultivar ‘CDC Imagine’, there were no differences among fungicide application timing treatments at stem elongation and early milk compared with the unsprayed control (75.9 kg hL^{-1}). Although, TW of the fungicide applied at mid-flower treatment (77.4 kg hL^{-1}) was greater than the unsprayed control and similar to the multiple application treatment (77.5 kg hL^{-1}). Differences between the unsprayed control (76.1 kg hL^{-1}) and the multiple application treatment (77.3 kg hL^{-1}) were detected for the cultivar ‘Lillian’.

Protein content (%)

For this group, there was an interaction between application timing and cultivar for protein content ($P<0.0001$) (Table 3.6). The protein content for 'AC Barrie' was 15.7% for the multiple fungicide application treatment, 15.5% for the mid-flower treatment, and 15.1% for the stem elongation fungicide application timing, which were higher than the unsprayed treatment with a mean of 14.3% (Table 3.7). For the moderately resistant cultivar 'CDC Imagine', the multiple application treatment had a protein content of 15.7%, which differed from the unsprayed treatment at 15%. No differences among application timings were detected for the cultivar 'Lillian' with protein content varying only between 16.6 and 16.8% (Table 3.6).

Table 3.7. High stripe rust severity site-years group at the mid-May seeding date and effect of fungicide application timing, wheat cultivar and their interaction on stripe rust severity, yield, thousand-kernel weight (TKW), test weight (TW) and protein content. Cultivars: 'AC Barrie' (stripe rust susceptible), 'CDC Imagine' (moderately resistant) and 'Lillian' (resistant); fungicide application timing: unsprayed, stem elongation (GS 31), mid-flower (GS 65), early milk (GS 73), and multiple application control (three fungicide applications, GS 31, 65 and 73)

Cultivar	Variable/treatment	Unsprayed	Stem elongation	Mid-flower	Early milk	Multiple application
'AC Barrie'	Stripe rust severity (%)	87.3 a	58.3 b	26.3 c	77.4 ab	11.4 d
	Yield (kg/ha)	2733 c	3595 b	4347 a	3091 bc	4911 a
	TKW (g)	32.6 c	35.6 b	38.2 a	34.9 bc	39.3 a
	TW (kg/hL)	77.5 c	78.8 b	79.9 a	78.8 b	80.1 a
	Protein content (%)	14.3 b	15.1 a	15.5 a	14.3 b	15.7 a
CDC Imagine	Stripe rust severity (%)	54.4 a	28.4 bc	14.3 cd	37.6 ab	4.2 d
	Yield (kg/ha)	2966 c	3362 bc	3914 ab	3365 bc	4248 a
	TKW (g)	35.7 b	36.7 b	38.0 a	36.3 b	38.7 a
	TW (kg/hL)	75.9 b	76.1 b	77.4 a	76.8 ab	77.5 a
	Protein content (%)	15.0 b	15.6 ab	15.4 ab	15.1 b	15.7 a
Lillian	Stripe rust severity (%)	1.3 a	0.5 a	0.6 a	0.1 a	0.8 a
	Yield (kg/ha)	3870 a	3930 a	4282 a	3782 a	4458 a
	TKW (g)	37.4 b	37.6 ab	38.7 a	37.9 ab	39.3 a
	TW (kg/hL)	76.1 b	76.2 b	77.0ab	76.7 ab	77.3 a
	Protein content (%)	16.6 a	16.8 a	16.6 a	16.7 a	16.8 a

Note: treatment means followed by the same letter in each row are not significantly different according to Tukey's test (P>0.05).

Table 3.8. High stripe rust severity site-years at the mid-May seeding date and effect of fungicide application timing and wheat cultivar on leaf spot severity. Cultivars: ‘AC Barrie’ (stripe rust susceptible), ‘CDC Imagine’ (moderately resistant) and ‘Lillian’ (resistant); fungicide application timing: unsprayed, stem elongation (GS 31), mid-flower (GS 65), early milk (GS 73), and multiple application control (three fungicide applications, GS 31, 65 and 73).

Treatment/Cultivar	**Leaf spot severity (%)**
Fungicide application timing	
Unsprayed control	25.1 a
Stem elongation	21.6 a
Mid-flower	11.1 b
Early milk	25.4 a
Multiple application	8.5 b
Cultivar	
‘AC Barrie’	18.2 ab
‘CDC Imagine’	14.9 b
‘Lillian’	21.9 a

Note: treatment means followed by the same letter are not significantly different according to Tukey’s test ($P>0.05$).

3.4.2.2 Low stripe rust severity group

Stripe rust severity (%)

For the low group of site-years with little stripe rust severity there was no statistical interaction between cultivar and application timing (P=0.2555) or differences among cultivars (P=0.2360), although differences among application timings were detected (P=0.0445) for stripe rust severity (Table 3.6). Stripe rust severity for these site-years was low and at some site-years no symptoms were observed, the average was 5% for the unsprayed treatment (mean of the three cultivars), which was not different from the stem elongation and early milk treatments (Table 3.9). The mid-flower fungicide application differed from the unsprayed treatment from 5% to 0.3% stripe rust severity. No stripe rust symptoms were observed in the multiple application treatment.

Leaf spot disease severity (%)

There were no differences among cultivars (P=0.5421) and no interaction between cultivar and application timing (P=0.7214) for leaf spot severity (Table 3.6). There were differences among application timings (P=0.0106), but only between the unsprayed (5%) and multiple application treatment (10%). None of the other fungicide applications differed from the unsprayed treatment (Table 3.9).

Yield effect (kg/ha)

No interaction was detected between cultivar and application timing (P=0.9482); however, differences among application timings (P=0.0003) and among cultivars (P=0.0168) were observed for this group (Table 3.6). Among fungicide application timings, the unsprayed treatment had lower yield (3857 kg/ha) than fungicide treatments at mid-flower (4325 kg/ha), early milk (4286 kg/ha) and the multiple application treatment (4484 kg/ha), none of which differed from each other (Table 3.9). The stem elongation timing did not differ from the unsprayed. Furthermore, 'AC Barrie' had a greater yield (4299 kg/ha) than 'CDC Imagine' (4089 kg/ha), but 'CDC Imagine' did not differ from 'Lillian' (4180 kg/ha).

Thousand kernel weight (g)

There was no interaction between cultivar and application timing (P=0.6084) (Table 3.6). Differences were detected among application timings (P<0.0001) and among wheat cultivars (P=0.0348). The TKW for the unsprayed treatment was 37.4 g, which differed from fungicide applied at mid-flower (38.5 g), fungicide applied at early milk stage (39 g) and the multiple application treatment (39.3 g) (Table 3.9). The stripe rust resistant cultivar 'Lillian' had higher TKW (38.7 g) than the moderately resistant cultivar 'CDC Imagine' (38.2 g), but neither 'Lillian', nor 'CDC Imagine' differed from 'AC Barrie' (38.4 g).

Test weight (kg/hL)

No interaction between cultivar and application timing (P=0.7544) for TW was detected in the low group seeded in mid-May (Table 3.6); however, differences among timings (P=0.0004) and among cultivars (P<0.0001) were observed for this group. Among the fungicide application timings, the unsprayed treatment had lower TW (79.2 g) than fungicide treatment at mid-flower (79.9 g) or the multiple application treatment (79.7 g), which did not differ from each other or the early milk treatment (79.7 g) (Table

3.9). The stem elongation timing (79.4 g) did not differ from the unsprayed control or the other fungicide application timings except for the multiple application treatment. Moreover, ‘AC Barrie’ had a greater TW (80.7 g) than ‘CDC Imagine’ (78.9 g) and ‘Lillian’ (79.3 g).

Protein content (%)

For this group, there were no differences among fungicide application timings (P=0.0695) and no interaction between cultivar and application timing (P=0.8097) for protein content (Table 3.6). There were differences among cultivars (P<0.0001); the protein content of ‘Lillian’ was 15.5%, which was higher than that of ‘CDC Imagine’ (14.5%) or ‘AC Barrie’ (14.4%) (Table 3.9).

Table 3.9. Low stripe rust severity site-years at the mid-May seeding date and effect of fungicide application timing and wheat cultivar on stripe rust severity, leaf spot disease severity, yield, thousand kernel weight (TKW), test weight (TW) and protein content. Cultivars: ‘AC Barrie’ (stripe rust susceptible), ‘CDC Imagine’ (moderately resistant) and ‘Lillian’ (resistant); fungicide application timing: unsprayed control, stem elongation (GS 31), mid-flower (GS 65), early milk (GS 73), and multiple application treatment (three fungicide applications, GS 31, 65 and 73).

Treatment/Cultivar	Stripe rust severity (%)	Leaf spot severity (%)	Yield (kg/ha)	TKW (g)	TW (kg/hL)	Protein content (%)
Fungicide application timing						
Unsprayed	4.9 a	10.4 a	3857 c	37.4 c	79.2 c	15.0 a
Stem elongation	3.8 a	7.8 ab	3993 bc	37.9 bc	79.4 bc	15.4 a
Mid-flower	0.3 b	7.3 ab	4325 a	38.5 ab	79.9 ab	15.6 a
Early milk	2.3 ab	7.1 ab	4286 ab	39.0 a	79.7 abc	15.2 a
Multiple application	0 b	4.5 b	4484 a	39.3 a	80.1 a	15.4 a
Cultivar						
‘AC Barrie’	ns	ns	4299 a	38.4 ab	80.7 a	14.4 b
‘CDC Imagine’	ns	ns	4089 b	38.2 b	78.9 b	14.5 b
‘Lillian’	ns	ns	4180 ab	38.7 a	79.3 b	15.5 a

Note: means followed by the same letter in each column do not differ significantly according to Tukey's test ($P \geq 0.05$). ns: not significant.

3.4.2.3 Correlations between variables measured at the mid-May seeding date

Pearson's correlation coefficients were calculated using the CORR procedure of SAS for the two groups at the mid-May seeding date.

High stripe rust severity site-years

For the high group, Pearson's correlation coefficients indicated a moderate inverse correlation between stripe rust severity and yield ($r=-0.4534$), TKW ($r=-0.6390$) and protein content ($r=-0.70804$) all at $P<0.0001$. However, the correlations were not significant between stripe rust severity and leaf spot severity ($P=0.6842$) or between stripe rust severity and TW ($P=0.2765$).

Low stripe rust severity site-years

Pearson's correlation coefficients were not significant between stripe rust severity and leaf spot severity ($P=0.7469$), yield ($P=0.6705$), TKW ($P=0.865$), TW ($P=0.0745$) or protein content ($P=0.1686$).

3.4.3 Early June seeding date

Based on the stripe rust severity of the control treatment (unsprayed susceptible cultivar, 'AC Barrie'), site-years were classified into 'high' or 'low' stripe rust severity groups (Table 3.10). The mean stripe rust severity of individual site-years comprising the high group ranged from 60 to 97%. Site-years where the control treatment was 10% or lower (no symptoms detected at Melfort) were classified as low stripe rust severity site-years. Disease severity for the high group was 87% for the control treatment, and 10% in the low group.

Table 3.10. High and low stripe rust severity site-year groups based on severity of the unsprayed treatment of ‘AC Barrie’ (susceptible) for the early June seeding date experiments.

Group	Site-Year
High stripe rust severity	Saskatoon 2013, 2014 and 2016
	Pike Lake 2014 and 2016
Low stripe rust severity	Saskatoon 2012 and 2015
	Pike Lake 2013 and 2015
	Melfort 2013 and 2014

3.4.3.1 High stripe rust severity group

Stripe rust severity (%)

There was an interaction between cultivar and application timing (P=<0.0001) for stripe rust severity (Table 3.6). The susceptible cultivar ‘AC Barrie’ had a mean stripe rust severity of 87% for the unsprayed control (Table 3.11). Stripe rust severity was reduced to 51% on ‘AC Barrie’ when fungicide was applied at mid-flower and to 54% when the fungicide was applied at stem elongation. As expected, the multiple application treatment had the lowest disease severity at 12%. The moderately resistant cultivar, ‘CDC Imagine’, had a mean stripe rust severity of 31% for the unsprayed treatment and a reduction to 20% was observed when fungicide was applied at stem elongation and to 14% when fungicide was applied at mid-flower. Stripe rust severity was very low for the resistant cultivar 'Lilian' for all fungicide treatments. The unsprayed control had a disease severity of 0.2% and fungicide treatments did not differ statistically from the unsprayed treatment.

Leaf spot disease severity (%)

There was no interaction between cultivar and application timing (P=0.1670) or among cultivars (P=0.3507) for leaf spot severity (Table 3.6). Differences among application timings were detected (P<.0001). The unsprayed treatment had the highest leaf spot severity at 22%; when fungicide was applied at stem elongation, the mean disease level was 16% and at mid-flower 13% (Table 3.12). The multiple application treatment had a disease severity of 10%.

Yield response (kg/ha)

For this group, there was an interaction between application timing and cultivar (P=<0.0001) for yield of wheat (Table 3.6). The yield of 'AC Barrie' was 3576 kg/ha when fungicide was applied at stem elongation, a 53% increase over the unsprayed treatment (2342 kg/ha) (Table 3.11). When the fungicide was applied at mid-flower a yield increase of 46% was observed and the yield for this treatment was 3429 kg/ha. As expected, the multiple application treatment had the highest yield at 4334 kg/ha. No statistical differences among fungicide application timing treatments at stem elongation, mid-flower and early milk and the unsprayed treatment were detected for 'CDC Imagine', although the yield of the treatment when fungicide was applied at mid-flower (3767 kg/ha) appeared to be much greater than the unsprayed treatment (3202 kg/ha).

The multiple application treatment had a yield of 4191 kg/ha and was significantly higher than the other treatments. No differences among fungicide treatments were detected for the cultivar Lillian.

Thousand kernel weight (g)

For this group there was an interaction between cultivar and fungicide application timing (P<0.0001) for TKW (Table 3.6). The TKW of the stripe rust susceptible cultivar 'AC Barrie' had a mean of 30.5 g for the unsprayed treatment, which was greatly increased when the fungicide was applied at mid-flower (35.3 g) and by the multiple application treatment (37.7 g) (Table 3.11). Fungicide treatments at the early milk stage of wheat (32.5 g) and stem elongation (33.9 g) were similar to the unsprayed treatment. The multiple application treatment for 'CDC Imagine' had a TKW of 36.4 g, which differed from the fungicide application treatments at stem elongation (35.1 g), early milk (35.2 g), mid-flower (36 g) and the unsprayed treatment (34.6 g). For 'Lillian', the TKW for the unsprayed treatment of 35.9 g differed only from the multiple application treatment at 38 g.

Test weight (kg hL^{-1})

There was no interaction between wheat cultivar and application timing (P=0.1614) for TW; however, differences among timings (P=0.0161) and among cultivars (P<0.0001) were observed (Table 3.6). Among the fungicide application timings, the unsprayed treatment had a TW of 77.3 kg/hL^{-1}, which was slightly less than fungicide treatment at mid-flower (77.9 kg/hL^{-1}) (Table 3.12). Furthermore, 'AC Barrie' had greater TW (79.1 kg/hL^{-1}) than 'CDC Imagine' (76.6 kg/hL^{-1}) or 'Lillian' (76.7 kg/hL^{-1}).

Protein content (%)

For the high group, there was an interaction between cultivar and fungicide application timing (P=0.0019) for protein content of wheat (Table 3.6). For ‘AC Barrie’, the unsprayed treatment had the lowest protein content at 13.1%, compared with fungicide application timings at mid-flower or stem elongation (both 13.9%) (Table 3.11). The multiple application treatment had the highest protein content at 14.8%. For ‘CDC Imagine’, the multiple application treatment was 14.6% and differed only from the unsprayed treatment at 13.5%. No differences among fungicide application treatments at stem elongation (14.3%), mid-flower (14.2%) or early milk (14.2%) wheat growth stages nor from the unsprayed treatment were detected. Furthermore, for the resistant cultivar ‘Lillian’ no difference among application timings were detected.

Table 3.11. High stripe rust severity site-years at the early June seeding date and effect of fungicide application timing, wheat cultivar and their interaction on stripe rust severity, yield, thousand-kernel weight (TKW) and protein content. Cultivars: 'AC Barrie' (stripe rust susceptible), 'CDC Imagine' (moderately resistant) and 'Lillian' (resistant); fungicide application timing: unsprayed treatment, stem elongation (GS 31), mid-flower (GS 65), early milk (GS 73), and multiple application control (three fungicide applications, GS 31, 65 and 73).

Cultivar	Variable	Unsprayed control	Stem elongation	Mid-flower	Early milk	Multiple application
'AC Barrie'	Stripe rust severity (%)	87.1 a	54.2 b	50.6 bc	86.8 a	11.5 c
	Yield (kg/ha)	2342 d	3576 b	3429 bc	2788 cd	4334 a
	TKW (g)	30.5 c	33.9 bc	35.3 b	32.5 c	37.7 a
	Protein content (%)	13.1 c	13.9 b	13.9 b	13.1 c	14.8 a
'CDC Imagine'	Stripe rust severity (%)	30.9 ab	20.2 ab	13.7 bc	33.2 a	4.5 c
	Yield (kg/ha)	3202 b	3525 ab	3767 ab	3315 b	4192 a
	TKW (g)	34.6 b	35.1 b	36.0 ab	35.2 b	36.4 a
	Protein content (%)	13.5 b	14.3 ab	14.2 ab	14.2 ab	14.6 a
'Lillian'	Stripe rust severity (%)	0.2 a	0.2 a	0 a	0.2 a	0 a
	Yield (kg/ha)	3339 a	3329 a	3561 a	3439 a	3741 a
	TKW (g)	35.9 b	36.6 ab	37.5 ab	36.8 ab	38.0 a
	Protein content (%)	15.3 a	15.6 a	15.4 a	16.0 a	15.9 a

Note: treatment means followed by the same letter in each row are not significantly different according to Tukey's test (P>0.05).

Table 3.12. High stripe rust severity site-years at the early June seeding date and effect of fungicide application timing and wheat cultivars on leaf spot disease severity and test weight (TW). Cultivars: 'AC Barrie' (stripe rust susceptible), 'CDC Imagine' (moderately resistant) and 'Lillian' (resistant); fungicide application timing: unsprayed treatment, stem elongation (GS 31), mid-flower (GS 65), early milk (GS 73), and multiple application treatment (three fungicide applications, GS 31, 65 and 73)

Treatment/Cultivar	Leaf spot severity (%)	TW (kg/hL)
Fungicide application timing		
Unsprayed	21.8 a	77.3 b
Stem elongation	15.8 b	77.3 b
Mid-flower	13.3 bc	77.9 a
Early milk	17.5 ab	77.0 ab
Multiple application	9.9 c	77.8 ab
Cultivar		
'AC Barrie'	ns	79.1 a
'CDC Imagine'	ns	76.6 b
'Lillian'	ns	76.7 b

Note: means followed by the same letter in each column are not significantly different according to Tukey's test (*P*>0.05). ns: not significant.

3.4.3.2 Low stripe rust severity group

Stripe rust severity (%)

In the low group there were differences among fungicide application timings and among cultivars (P<0.0001) for stripe rust severity (Table 3.6). The unsprayed treatment for 'AC Barrie' had 10% stripe rust, which was reduced when fungicide was applied at mid-flower (1%) and at stem elongation (2%) (Table 3.13). For the moderately resistant cultivar, 'CDC Imagine', low stripe rust severity was observed in the unsprayed control (3%) and decreased with the fungicide application timings (to 1% and 0%). No stripe rust was observed on the resistant cultivar 'Lillian'.

Table 3.13. Low stripe rust severity site-years at the early June seeding date and effect of fungicide application timing, wheat cultivar and their interaction on stripe rust severity (%). Cultivars: 'AC Barrie' (stripe rust susceptible), 'CDC Imagine' (moderately resistant) and 'Lillian' (resistant); fungicide application timing: unsprayed treatment, stem elongation (GS 31), mid-flower (GS 65), early milk (GS 73), and multiple application control (three fungicide applications, GS 31, 65 and 73).

Cultivar	Unsprayed	Stem elongation	Mid-flower	Early milk	Multiple application
'AC Barrie'	9.8 a	2.4 c	1.0 cd	7.5 ab	0.3 d
'CDC Imagine'	2.8 a	0.7 b	0.1 b	2.7 a	0.1 b
'Lillian'	0.1 a	0.6 a	0.1 a	0.1 a	0.2 a

Note: treatment means followed by the same letter in each row are not significantly different according to Tukey's test (*P*>0.05).

Leaf spot disease severity (%)

No differences among cultivars (P=0.2077) and no interaction between cultivar and application timing were observed (P=0.3727) for leaf spot severity (Table 3.6). Differences among fungicide application timings between unsprayed and multiple application treatment were detected (P=0.0204) (Table 3.14).

Yield response (kg ha^{-1})

No interaction between cultivar and application timing was detected (P=0.9042). Differences among fungicide application timings (P=0.0028) and among wheat cultivars (P=0.0033) were observed (Table 3.6). No differences were detected between the unsprayed treatment (3443 kg ha^{-1}), and the fungicide applications timings at stem elongation (3747 kg ha^{-1}), mid-flower (3908 kg ha^{-1}) or early milk (3647 kg ha^{-1}); however, the unsprayed treatment did differ from the multiple application treatment (4285 kg ha^{-1}). The yield of 'AC Barrie' (4023 kg ha^{-1}) was higher than 'Lillian' (3599 kg ha^{-1}), but not different from' CDC Imagine' (3796 kg ha^{-1}) (Table 3.14).

Thousand kernel weight (g)

There were no interactions between cultivar and application timing (P=0.2478) for TKW. Differences among application timings and among wheat cultivars (P<0.0001) were detected (Table 3.6). The TKW for the unsprayed treatment (36.4 g) did not differ from fungicide application at stem elongation (37.3 g) or at early milk (37.2 g). The multiple application treatment (39.1 g) and the application at mid-flower (37.8 g) differed from the unsprayed treatment, however. Additionally, the susceptible cultivar

‘AC Barrie’ and the resistant cultivar ‘Lillian’ had higher TKW at 37.8 g compared with ‘CDC Imagine’ 37 g (Table 3.14).

Test weight (kg hL^{-1})

No interaction between cultivar and application timing (P=0.9383) was detected for TW; however, differences among timings and among cultivars (P<0.0001) were observed for the high group (Table 3.6). Among the fungicide application timings, the unsprayed treatment had lower TW (78 kg hL^{-1}) than fungicide treatments at mid-flower (78.8 kg hL^{-1}) or the multiple application treatment (79.3 kg hL^{-1}). Additionally, ‘AC Barrie’ had greater TW (80 kg/hL) than ‘CDC Imagine’ (77.6 kg hL^{-1}), and both differed from ‘Lillian’ (78.3 kg hL^{-1}) (Table 3.14).

Protein content (%)

No interaction between cultivar and application timing (P=0.3022) for protein content was detected (Table 3.6); however, differences among wheat cultivars (P<.0001) and fungicide application timings (P=0.001) were observed. The unsprayed treatment at 14.7% differed only from the multiple application treatment at 15.6%; no differences among the application timings at stem elongation (15.3%), mid-flower (15.3%) and early milk (15.1%) were detected. Additionally, the cultivar ‘Lillian’ had a high protein content of 15.6% compared with the other two cultivars both at 15% (Table 3.14).

Table 3.14 Low stripe rust severity site-years at the early June seeding date and effect of fungicide application timing and wheat cultivar on leaf spot disease severity, yield, thousand kernel weight (TKW), test weight (TW) and protein content. Cultivars: 'AC Barrie' (stripe rust susceptible), 'CDC Imagine' (moderately resistant) and 'Lillian' (resistant); fungicide application timing: unsprayed treatment, stem elongation (GS 31), mid-flower (GS 65), early milk (GS 73), and multiple application treatment (three fungicide applications, GS 31, 65 and 73).

Treatment/Cultivar	Leaf spot severity (%)	Yield (kg/ha)	TKW (g)	TW (kg/hL)	Protein content (%)
Fungicide application timing					
Unsprayed	16.7 ab	3443 b	36.4 c	78.0 c	14.7 b
Stem elongation	18.9 a	3747 ab	37.3 bc	78.6 bc	15.3 ab
Mid-flower	11.4 b	3908 ab	37.8 b	78.8 b	15.3 ab
Early milk	19.8 a	3647 b	37.2 bc	78.5 bc	15.1 ab
Multiple application	12.9 b	4285 a	39.1 a	79.3 a	15.6 a
Cultivar					
'AC Barrie'	ns	4023 a	37.8 a	80.0 a	15.0 b
'CDC Imagine'	ns	3796 ab	37.0 b	77.6 c	15.0 b
'Lillian'	ns	3599 b	37.8 a	78.3 b	15.6 a

Note: means with the same letter in each column are not significantly different according to Tukey's test (*P*>0.05). ns: no significant effect

3.4.3.3 Correlations among variables measured at the early June seeding date

Pearson's correlation coefficients were calculated using the CORR procedure of SAS for the two groups at the early June seeding date.

High stripe rust severity

For the high group the Pearson's correlation coefficient indicated a weak to moderate inverse correlation between stripe rust severity and yield (r=-0.32224, P<0.0001), and a moderate to strong negative correlation between stripe rust severity and TKW (r=-0.65179; P<0.0001) and between stripe rust severity and protein content (r=-0.50106, P<0.0001) and there was a low correlation between stripe rust and TW (r=0.28208, P<0.0001). There was no correlation between stripe rust severity and leaf spot severity (P=0.2036).

Low stripe rust severity

For this group the Pearson's correlation coefficients suggested a weak to moderate inverse correlation between stripe rust severity and yield (r=-0.3209, P<0.0001) and a low correlation between stripe rust with TKW (r==0.26066, P<0.0001) There was no correlation between stripe rust severity and leaf spot severity (P=0.3173), stripe rust severity with TW (P=0.5977) or stripe rust severity with protein content (P=0.1686).

3.5 Discussion

Stripe rust of wheat has a detrimental effect on yield and quality when susceptible cultivars are grown (Chen 2014). The use of DMI fungicides (Group 3) can reduce disease severity and increase yield in wheat cultivars when applied alone or when more than one active ingredient within that group are combined, such as tebuconazole, prothioconazole and propiconazole (Jorgensen et al. 2018, Lopez et al. 2015, Barro et al 2017). The application of tebuconazole alone can offer a high level of protection of the leaf area due to its slow degradation, thus providing a long period of plant protection from leaf diseases (Lehoczki-Krsjak 2013). This was observed in my field trials when tebuconazole was applied at site years with high stripe rust severity. Both stripe rust and leaf spot severity were reduced compared with the unsprayed treatment of each cultivar. However, fungicide application treatments had no effect on stripe rust severity, but some effect was shown on yield when disease pressure was low or at trace levels. It was observed that at site-years with low disease pressure, environmental factors, such as precipitation were below normal and temperatures above normal during the months of May to July. Conditions such as warm nights and low humidity likely played a role at site-years with low stripe rust severity during the growing season (Chen, 2005).

If environmental conditions are conducive for the development of stripe rust during the field season, yield losses may occur on susceptible and moderately resistant cultivars without fungicide application. Several studies have demonstrated this. For example, in 40 field trials over 2 years in 10 European countries, fungicide application of four azole fungicides were made on winter wheat to control Septoria leaf blotch, stripe rust and leaf rust (Jorgensen et al. 2018). The fungicides applied at the flag leaf stage provided effective control of stripe rust (80 to 90% reduction in symptoms) in 10 site-years when the disease was present, and yield increased between 19 to 32% as a result of the treatments. In South Africa in the late 1990s, fungicide application of tebuconazole and propiconazole at the flag leaf stage to control stripe rust in winter and spring wheat increased yield from 7 to 56% on stripe rust susceptible wheat cultivars (Boshoff et al. 2002). My results support previous reports that fungicides applied on the susceptible or moderately resistant wheat cultivars improved yield by 32 to 60% compared with the unsprayed control when stripe rust severity was high.

The crop growth stage at the time of fungicide application and the number of fungicide applications were critical to reduced stripe rust severity in my study. When stripe rust severity was high, a single fungicide application made at mid-flower or at stem elongation growth stages (GS 65 - GS31) to the susceptible cultivar 'AC Barrie' or to the moderately resistant cultivar 'CDC Imagine' greatly reduced severity of stripe rust. This effect was greater at the mid-May seeding date compared with the early June seeding date. When the fungicide was applied to the stripe rust resistant cultivar 'Lillian' in high stripe rust severity site-years seeded mid-May, there was no effect on stripe rust at either growth stage because little disease occurred. However, yield of 'Lillian' was increased when the fungicide was applied compared with the unsprayed control. This can be explained by the leaf spots that occurred on this stripe rust resistant cultivar, which happened to be moderately susceptible to leaf spots.

The multiple application treatment reduced stripe rust symptoms on the susceptible and moderately resistant cultivars in the site-years with high stripe rust severity at either seeding date. However, whether this increase would be economically beneficial for a farmer requires further economic study to investigate the return on investment for more than a single application of fungicide. Multiple applications would have a negative economic impact for growers under low stripe rust or leaf spot severity. In Alberta, Kumar et al. (2019), reported that a single fungicide application of propiconazole (Tilt®) to wheat when stripe rust was observed at late heading stage (GS59) generally reduced stripe rust symptoms and increased yield under high to moderate levels of disease severity on susceptible cultivars in naturally infected field tests. In Portugal, Gomes et al. (2018) applied a foliar fungicide (a.i. bixafen and prothioconazole) twice, at stem elongation (GS31) and at booting stage (GS44) and reported reduced stripe rust severity and increased yield of up to 70% on susceptible wheat cultivars. However, if the fungicide was applied to cultivars with a moderately resistant and /or slow rusting response, a single fungicide application at stem elongation (GS34) was enough to control stripe rust and increase yield.

Most wheat produced is for human consumption; thus, quality is an important factor (Shewry and Hey 2015). The main effect of a foliar fungicide is to maintain green leaf area, which is critical for kernel development and grain fill, and desirable for flour extraction in milling (Rosewarne et al. 2006). In my study, both TKW and TW were improved when the fungicide was applied at both stem elongation and at mid-flower growth stages on the susceptible stripe rust cultivar 'AC Barrie' and on the moderately resistant cultivar 'CDC Imagine' in site-years where disease pressure was high. These seed quality factors were improved even when stripe rust pressure was low at both seeding dates. A similar trend was reported by Chen (2014), with an increase in TW of close to 2% on spring wheat cultivars after fungicide application at flowering and boot stages. Kumar et al. (2019) also reported increased TKW and TW when fungicide was applied to susceptible and moderately resistant cultivars. Similarly, fungicide application to control stripe rust on the susceptible cultivar increased protein content at both seeding dates compared with the unsprayed control. This increase in protein content was observed when the fungicide was applied at mid-flower and at stem elongation stages when disease pressure was high. However, for the moderately resistant and resistant cultivar protein content did not differ.

The cultivar response to fungicide application largely depended on the stripe rust resistance level of the cultivar. For instance, the stripe rust susceptible cultivar 'AC Barrie' had the largest responses to fungicide in terms of reduction of stripe rust severity and improvement in yield and quality. This cultivar does not have any known stripe rust resistance genes, but it was one of the most popular CWRS cultivars grown in the prairies for many years because of its high yield and protein content, and because growers appreciated its short, strong straw (McCallum and DePauw 2008). The cultivar 'CDC Imagine' has moderate resistance to stripe rust; however, there was an increase in yield and quality when fungicide was applied, although not as much as for 'AC Barrie'. Cultivar 'CDC Imagine' carries the important adult plant resistance gene *Yr18,* which confers partial resistance to stripe rust and has been widely used in Canadian cultivars and breeding programs (McCallum et al. 2012). This pleiotropic APR gene is also known as *Lr34*, *Sr57* and *Pm38* because it confers resistance to other pathogens including *Puccinia triticina*, *Puccinia graminis f. sp. tritici* and *Erysiphe graminis* causal pathogens of leaf rust and powdery mildew. Resistance is enhanced when *Yr18* interacts with other unlinked genes as is the case for the resistant cultivar “Lillian", which carries the APR genes *Yr18* and *Yr36* (Randhawa et al. 2012). In addition, some researchers have suggested that this resistant cultivar may carry other unknown resistance genes against the most prevalent races in western Canada (Brar and Kutcher 2016). The gene *Yr36* has been linked to the high grain protein content gene *Gpc-B1*, which could explain the protein content in my study. Even though the protein levels of the stripe rust susceptible and moderately resistant cultivars increased when the fungicide was applied, the protein content of the resistant cultivar ‘Lillian’ was highest.

The use of a fungicide to control stripe rust of wheat was affected by seeding date in this study. When wheat was seeded in mid-May and stripe rust infection was severe, the effect on yield and stripe rust severity was greatest if the fungicide was applied at the mid-flower stage on susceptible and moderately resistant wheat cultivars. These results agree with De Wolf et al. (2012) from Kansas, who stated that foliar fungicide applications in between flag leaf emergence and anthesis (flowering) growth stages to control stripe rust are effective because it is at these stages that the plant uses energy for grain development and yield formation. However, in my study, when the wheat was planted late (early June) and stripe rust severity was high, fungicide application at stem elongation had the same effect as an application at mid-flower stage. This effect can be attributed to an early onset of the disease in relation to crop growth stage; the severity of stripe rust infection on the lower leaves of wheat planted in early June was higher than on plants seeded in mid-May. These observations from our field study are in line with Braithwaite et al. (1998), who indicate that if stripe rust was observed after heading the most effective time to control stripe rust was at the flowering stage (GS65) rather than at the stem elongation stage. This indicates that the time of onset of stripe rust is critical when defining the optimum growth stage for an effective fungicide application to control the disease.

In summary, this study demonstrated that the application of a foliar fungicide may be beneficial to protect moderately resistant and susceptible cultivars when there is the potential for severe stripe rust. A single fungicide application at mid-flower on susceptible and moderately resistant bread wheat cultivars seeded in mid-May in central-Saskatchewan had beneficial effects on yield and other factors such as TW, TKW and protein content under high stripe rust severity conditions. However, if the same susceptible and moderately resistant cultivars were seeded in early June and there was high stripe rust severity, fungicide application timing was equally beneficial at the stem elongation and the mid-flower growth stages of wheat. There was no benefit to applying fungicide to the resistant cultivar regardless of seeding date. The use of resistant cultivars should be one of the main considerations for wheat growers before planting as the use of a fungicide should be avoided or limited to reduce input costs and impact on the environment. These results should be useful to help western Canadian wheat growers make practical decisions on when to apply fungicides to control stripe rust with consideration for seeding date, growth stage and environmental conditions, as well as the history of stripe rust occurrence on their farm.

CHAPTER 4

4. INHERITANCE OF RESISTANCE TO STRIPE RUST (CAUSED BY *Puccinia striiformis* f. sp. *tritici*) IN TWO CROSSES OF SPELT AND SPRING WHEAT

4.1 Introduction

Stripe rust caused by *Puccinia striiformis* f. sp. *tritici* is one of the most devastating diseases of wheat, particularly in cool and temperate areas of the world. Favorable environmental conditions for the development of stripe rust can significantly reduce yield and quality in susceptible wheat cultivars (Chen et al. 2002). The most common control measures are the use of resistant wheat cultivars and the application of foliar fungicides (Chen 2005).

Breeding for resistance is the most environmentally and economically effective approach to control stripe of wheat (Line and Chen 1995). However, it is an ongoing task to breed for stripe rust resistance because resistant cultivars usually do not remain resistant for long. The period of time a cultivar maintains effective resistance depends on the frequency of virulence shifts in the *Pst* populations. Currently, there are close to 80 officially designated *Yr* genes in wheat; some of these genes are expressed at all growth stages (ASR), and are race-specific and follow the gene-for-gene theory of host-pathogen interactions. Others are adult plant resistance genes (APR), which are expressed at the adult plant stage. This resistance is non-race specific (Cloutier 2007; McIntosh 1992). Rapid virulence changes in the pathogen population can overcome the resistance of cultivars and result in epidemics. Race characterization of 59 Pst isolates from western Canada by Brar and Kutcher (2016) found 33 races (31%), of which race C-PST-1 was the most frequently detected. It is avirulent on genes *Yr1, Yr5, Yr10, Yr15, Yr24/26, YrSP, YrTye*, and Hybrid 46; C-PST-2 was less frequently detected (7%) and was avirulent on *Yr1, Yr5, Yr10, Yr15, Yr24/26, Yr32, YrSP, YrSu, YrTye*, Hybrid 46, Nord Deprez, and Lillian.

Spelt (*Triticum aestivum* ssp. *spelta*) is one of the oldest sub-species of wheat; commercial cultivars of spelt have been developed based on its health benefits and high nutritional value. It has been included in varietal development breeding programs because it has a unique genetic composition that it makes it easy to cross with bread wheat. It is a source of important traits for bread wheat, such as high grain quality and resistance to pathogens such as *Pst* (Packa et al. 2019). The gene *Yr5* was derived from *T. spelta* var*. album*, a hexaploid wheat that has been reported to confer resistance to almost all isolates of *Pst* world-wide and its combination with other ASR and APR genes should provide effective and durable resistance to stripe rust (Kema 1992b).

4.2 Hypothesis and objectives

4.2.1 Hypothesis

Stripe rust resistance in the crosses of spelt *(T. aestivum* subp. *spelta)* with bread wheat (*T. aestivum)* is simply inherited.

4.2.2 Objective

Determine the inheritance of adult plant resistance to stripe rust derived from two spelt crosses x bread wheat under controlled conditions.

4.3 Materials and methods

4.3.1 Plant material

The experimental plant material comprised $F_{2:3}$ breeding lines obtained from two stripe rust resistant spring spelt genotypes ('CDC Silex' and 10Spelt17) and the susceptible stripe rust bread wheat cultivar 'Avocet'. The spelt parent lines had high yield, early maturity, resistance to leaf rust, and other inherent spelt wheat traits such as susceptibility to stem rust and the requirement for dehulling. The crosses were made in 2014. The F_1 generation was grown in 2015 and each plant harvested separately to form 6 populations per cross. Per population, 150-200 F_2 seeds were grown in the greenhouse and three weeks later hand transplanted to the field. Seventy-two F_2 adult plants were harvested individually per cross and screened for stripe rust resistance at the adult plant stage to verify segregation in the $F_{2:3}$ generation.

4.3.2 Inoculum production

From a set of *Pst* isolates collected from the Cereal and Flax Pathology program at the University of Saskatchewan, one isolate, W003, was selected to test the material. This isolate belongs to race C-PST-2, which is the second most frequently identified race in the Brar and Kutcher (2016) study. The isolate was collected in Kinley Saskatchewan in 2011 by the Cereals and Flax laboratory of the University of Saskatchewan.

The susceptible cultivar 'Avocet' was used for inoculum production. Nine kernels were sown in 10-inch pots containing potting mix and kept in a rust-free growth cabinet with a photoperiod of 16 hours and a day-time temperature of 21ºC and a night-time temperature of 16ºC. At the second leaf stage the plants were inoculated with a suspension of W003 urediniospores and mineral oil (Bayol®) inside gelatin caps to disperse the mix evenly across the leaf area using a compressed air inoculator and a small compressor. The plants were left to dry for 2 hours at room temperature and placed in a humidity chamber at 10ºC for 24 hours in the dark at 100% RH. Plants were then transferred to a growth chamber with a photoperiod of 16 hours and day and night temperatures of 17 and 10ºC, respectively. Sporulation occurred 14 days after inoculation and the spores were harvested daily by tapping the leaves gently over aluminum foil and dried for 24 hours in a desiccator with silica gel beads and stored in plastic vials at -80ºC until use. Once the urediniospores were ready to be used, they were heat-shocked in a water bath for 5 minutes at 40ºC to break dormancy.

4.3.3 Screening procedures

A total of 18 F_3 adult plants per cross/population were tested including the two spelt and bread wheat parental lines, '10Spelt17', 'CDC Silex" and 'Avocet', as well as four bread wheat cultivars varying in stripe rust resistance as controls, 'AC Barrie' (S), 'CDC Imagine' (MR), 'Lillian' (R) and 'Avocet-Yr5' (R). The material was sown in 1-gallon pots with three plants in each and arranged in the growth chamber with a photoperiod of 16 hours and 21 / 16ºC day/night temperatures. The flag and penultimate leaves were inoculated with 100 ul of spore suspension per leaf once the flag leaf was fully extended, approximately at booting stage (GS45) with the *Pst* isolate W003 using the procedure described previously.. Disease severity and infection type assessments were conducted weekly on each plant beginning when the susceptible checks showed symptoms of stripe rust, until the susceptible check reached 80 to 100% disease severity on the Modified Cobb's scale (Peterson et al., 1948) on both flag and penultimate leaves.

4.3.4 Data analysis

To determine whether the data fit the ratios for one or two loci conferring resistance to stripe rust, the Chi -Square test using the Yates correction equation was used to determine the goodness of fit.

4.4 Results

The parental spring spelt lines showed high resistance to isolate W003 with no or trace levels of stripe rust severity. The susceptible cultivars 'AC Barrie' and the parental line 'Avocet' had stripe rust severities of 85 and 89%, respectively. Based on CDC Imagine, which carried gene *Yr18* conferring moderate resistance to stripe rust, lines were accepted as resistant when the disease severity was ≤ 20% (Figure 4.1).

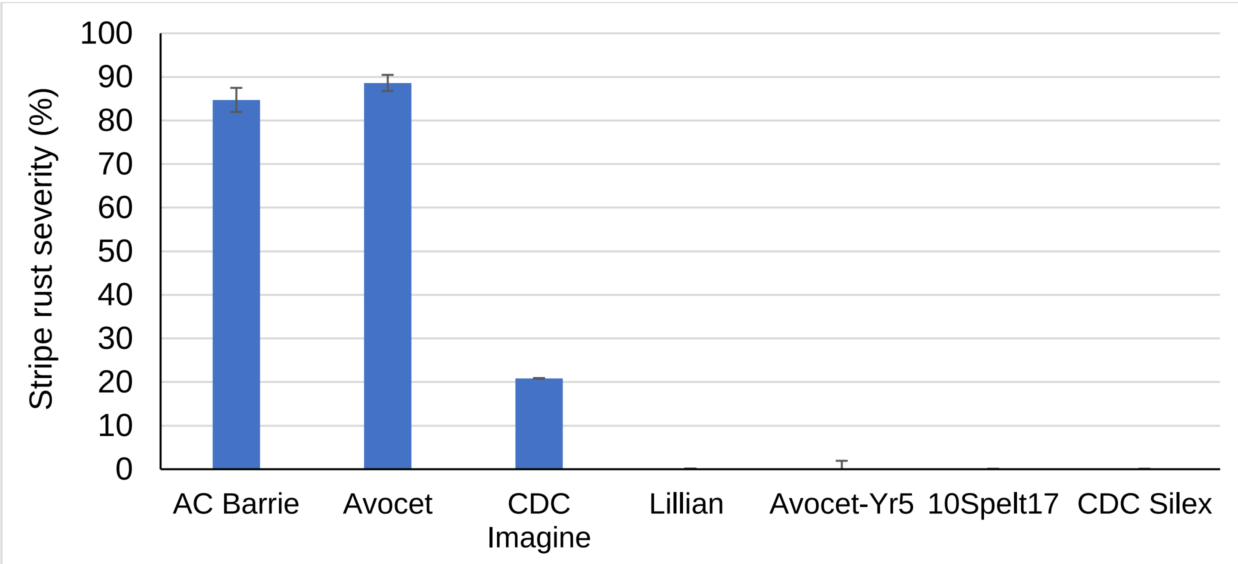

Fig. 4.1. Stripe rust severity of check cultivars and parental lines evaluated at the adult plant stage after inoculation with *Puccinia striiformis* f. sp. *tritici* race C-PST-2 (isolate W003).

The evaluation of the progenies in the $F_{2:3}$ generation for the crosses ‘Silex’ X ‘Avocet’ and ‘10Spelt17’X ‘Avocet’ exhibited stripe rust resistance to *Pst* race C-PST-2 (isolate W003) with observed population means of 69 and 71 showing some grade of resistance respectively (Table 4.1).

Table 4.1. Disease reaction of $F_{2:3}$ progenies from crosses of spelt lines (CDC Silex and 10Spelt17) and spring wheat (Avocet) to *Puccinia striiformis* f. sp. *tritici* race C-PST-2 (isolate W003); critical X^2 value = 3.8441.

Cross	Stripe rust reaction	Expected Ratio	Expected	Observed	Chi-Square	P value
Silex X Avocet	R[a]	3	54	69	17.241	<0.0001
	S[b]	1	18	3		
	Total			72		
	R	15	67.5	69	0.904	0.3417
	S	1	4.5	3		
	Total			72		
10Spelt17 X Avocet	R	3	54	71	22.555	<0.0001
	S	1	18	1		
	Total			72		
	R	15	67.5	71	3.688	0.0547
	S	1	4.5	1		
	Total			72		

[a] - resistant reaction; [b] - susceptible reaction.

Based on a significance level of 5% and critical Chi-square (X^2) value of 3.8441, stripe rust resistance in the crosses did not fit a 3:1 ratio but were consistent with a 15:1 ratio indicating that it is possible that there are at least two loci conferring adult plant resistance in these crosses of spring spelt and bread wheat.

4.5 Discussion

The use of resistant cultivars is the most effective and environmentally friendly method to control stripe rust. All stage resistance to stripe rust is non-durable and race-specific. Rapid development of new races of the pathogen can cause epidemics due to the narrow genetic diversity of most wheat cultivars (Gao et al. 2011). The use of other sources of resistance to stripe rust can increase diversity as is the case for spelt (*Triticum aestivum* ssp. *spelta*), which has the well known and excellent ASR gene, *Yr5*, that is avirulent to all known races of stripe rust in Canada (Yan et al. 2003).

This study demonstrated the nature of APR to stripe rust in the two spelt genotypes CDC Silex and 10Spelt17, which were crossed with a susceptible bread wheat cultivar. Few studies have determined the inheritance of adult plant stripe rust resistance in spelt. Kema (1992b) tested seven European and Iranian spelt wheat accessions hybridized with a susceptible bread wheat cultivar to study the genetics of resistance to stripe rust at the seedling stage. Six of these accessions tested with several *Pst* races at the F_1, F_2 and BC_1F_1 stages were found to carry a monogenic mode of resistance attributed to the gene*Yr5* from *T. aestivum* ssp. *spelta*. However, the response to each race was variable for some crosses in the F_2 revealing no susceptible plants, and in other cases segregation for two independent dominant resistance factors, similar to what I observed in my study. The inheritance of resistance will depend on the *Pst* race and the parents of the cross.

The goodness of fit of my $F_{2:3}$ lines to a 15:1 ratio suggests that the genotypes CDC Silex and 10Spelt 17 each possess two genes controlling stripe rust resistance to race C-PST-2. It is possible that the genotypes used in this study have a combination of other effective and non-race specific adult plant resistance genes. However, there is a need for further testing with additional races and different populations from these crosses to determine the genes conditioning this adult plant resistance to stripe rust.

CHAPTER 5

5 GENERAL DISCUSSION AND FUTURE WORK

Stripe rust of wheat can have a detrimental effect on yield and quality. This disease commonly occurs in spring and winter wheat cultivars grown in Saskatchewan when environmental conditions are conducive. Fungicide applications are an effective solution to control this disease when susceptible cultivars are grown (Chen 2014). The focus of this project was to determine the effect of tebuconazole fungicide applied to three bread wheat cultivars that varied in susceptibility to stripe rust from susceptible to resistant. Fungicide was applied at three growth stages and included an unsprayed control and a multiple application treatment. Two experiments that differed in seeding date (mid-May and early June) were established at each of 11 site-years. Site-years were divided into two groups (high and low) based on stripe rust severity of the unsprayed susceptible cultivar ‘AC Barrie’. When environmental conditions were conducive for the development of the disease, high stripe rust pressure was observed in the mid-May seeding date. A single fungicide application at mid-flower growth stage of wheat (GS65) decreased disease severity in the susceptible cultivar ‘AC Barrie’ and the moderately resistant cultivar ‘CDC Imagine’, compared with the unsprayed treatments of these cultivars. There was a significant increase in yield, test weight, thousand kernel weight and protein content of each cultivar. Furthermore, when the experiment was seeded in early June and there was high stripe rust pressure, a single fungicide application at stem elongation stage had the same effect as an application at mid-flower stage. Stripe rust severity was reduced on both the susceptible and moderately resistant cultivars; however, yield and quality were improved only for the susceptible cultivar, not the moderately resistant cultivar. This effect was attributed to the onset of the disease at early crop growth stages. At the same time, for the site-years where the disease pressure was low, fungicide application treatments had little effect on stripe rust symptoms or yield. Low stripe rust severity site-years were associated with low precipitation and higher than normal temperatures during the growing season, particularly the months of May to July.

For the resistant wheat cultivar ‘Lillian’ there was no effect of fungicide application due of the low levels of stripe rust in the field. However, there was an increase in yield, which can be explained by reduced leaf spot severity due to the fungicide application that occurred on this cultivar, which happens to be moderately susceptible to the leaf spot complex. The multiple fungicide application treatment had the greatest reduction in stripe rust severity, and increased grain yield and quality of the susceptible cultivar in the site-years with high stripe rust severity at either seeding date.

The cost of the fungicide tebuconazole is \$32.48 ha^{-1} for the product and approximately \$14.82 ha^{-1} for the application cost for a total of \$47.30 ha^{-1} (Jason McMartin, Bayer CropScience representative, personal communication April, 2021). In this study, the single fungicide application at mid-flowering and the multiple application treatment increased yield by 1614 and 2178 kg ha^{-1}, respectively compared with the unsprayed treatment for the susceptible cultivar 'AC Barrie' in the high stripe rust severity group when seeded in mid-May. Considering a wheat price of $262 per metric tonne, this resulted in additional income of $451.47 ha^{-1} and in the case of a multiple fungicide application an additional income of $581.87 ha^{-1}. Similarly, when the fungicide was applied at the stem elongation stage in the early June seeding date to the susceptible cultivar 'AC Barrie', yield increased by 1234 kg ha^{-1} compared with the unsprayed control, with additional income of $341.27 ha^{-1}. The multiple fungicide application treatment increased yield by 1992 kg ha^{-1} resulting in additional income of $527.93 ha^{-1}.

However, when the fungicide was applied to the moderately resistant cultivar 'CDC Imagine', multiple applications were not worth the cost compared with a single application at mid-flowering or at stem elongation. Therefore, a single fungicide application and multiple applications could be profitable when a susceptible bread wheat cultivar is grown and there is high disease pressure in the field. A single fungicide application may be cost effective when a moderately resistant cultivar is grown. However, these costs are associated only with the fungicide application so the net return from growing wheat depends on each farmer's situation and practices and other costs may need to be considered.

These results should be useful to develop recommendations for fungicide application timing and cultivar selection that will optimize economic returns for growers, and for growers to make practical and environmentally friendly decisions on when to apply fungicides to control stripe rust. Consideration should also be given to seeding date, growth stage and environmental conditions, as well as the history or risk of stripe rust on growers' farms.

The purpose of the second study in this thesis was to determine if the inheritance of stripe rust resistance of two spelt genotypes crossed with a susceptible bread wheat cultivar was simply inherited. Stripe rust resistance in the spelt genotypes examined indicated that the effective resistance observed at the adult plant stage was due to two genes. It is possible that the genotypes used in this study have a combination of other effective and non-race specific adult plant resistance genes. Additional *Pst* races from different locations in Canada should be tested at the seedling and adult stages for these two crosses in advanced generations. It would be interesting to characterize the disease resistance observed in this study using molecular markers to confirm that it is two genes conditioning resistance at the adult plant stage, or if the inheritance of resistance is more complex.

The use of a cultivar with a single ASR gene can be compromised due to the genetic variability of the pathogen. Using durable resistance, as is hypothesized to be the case for most APR genes to stripe rust, can be conditioned by additive and/or epistatic effects of multiple genes. Most breeding programs in western Canada are incorporating these APR genes into their germplasm because this resistance is expected to be durable. Farmer adoption of these newly developed cultivars could reduce fungicide costs by \$32.48.30 ha^{-1}or more. As observed in the previous fungicide study, the yield of the resistant cultivar 'Lillian' was 41% higher than the susceptible cultivar 'AC Barrie' when stripe rust was severe. Therefore, growing a resistant cultivar is the most cost-effective and environmentally friendly means to control stripe rust.

In the future, the study of fungicides to control stripe rust could include fungicides with different active ingredients and modes of action in addition to the single foliar fungicide used in this study, as well as a selection of more recently registered cultivars that vary in resistance to stripe rust. Finally, future research might include a more in-depth economic analysis of single and multiple fungicide applications on susceptible and moderately resistant cultivars.

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APPENDIX

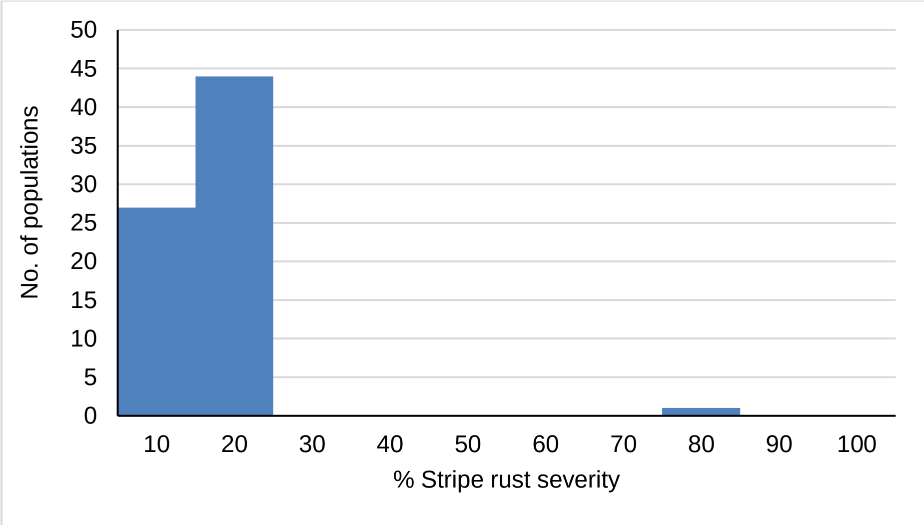

Fig A1. Distribution of stripe rust severity means in growth chamber for the cross 10spelt17 x Avocet, for the $F_{2:3}$ plants tested after inoculation with *Puccinia striiformis f.sp. tritici* race C-PST 2 (isolate W003). Severity scores are based on the modified Cobb scale (Peterson et al. 1948).

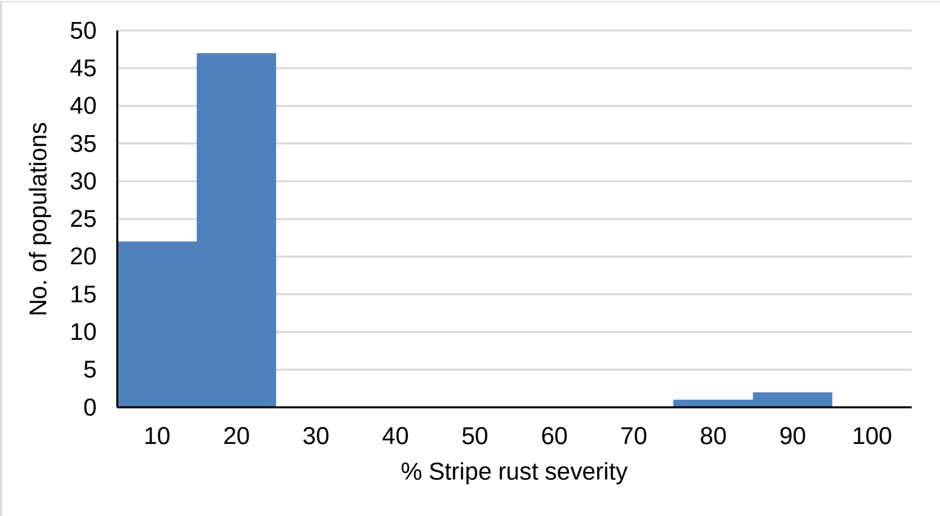

Fig A2. Distribution of stripe rust severity means in growth chamber for the cross CDC Silex x Avocet, for the total of $F_{2:3}$ plants tested after inoculation with *Puccinia striiformis f.sp. tritici* race C-Pst 2 (isolate W003). Severity scores are based on the modified Cobb scale (Peterson et al. 1948).